# SAFETY AND PHARMACOLOGICAL PROFILE OF *NAGA CHENDURAM*

The dissertation Submitted by

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## Chennai – 47

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# *INTRODUCTION*

# 1. INTRODUCTION

The siddha system dates back to5000 BC profounded by saint Agathiyar and his clan numbering 18 such Siddhars. This system is an amalgam of tamil literature, culture, tradition, health and many such living forms of 64 types.

The National Institute of Siddha was started on 2004 and the department of Gunapadam is also functioning on this date. So Many researches had been carried out for animal experimentation and drug profile.

The present study is about the safety and pharmacological profile of ***NAGA CHENDURAM*** on animal models.

Preclinical evaluation of acute and sub acute toxicity study was carried out in K.Kcollege of Pharmacy Gerugambakkam and sub-chronic toxicity study of the drug was carried out in animal house, NIS, Chennai.

The standard operative procedure has been followed and the drug labeling was also done. Review of the ingredients, chemical analysis shows sufficient molecules of therapeutic benefits for the safety and efficacy of the drug *Naga chenduram*.

Standardization includes organoleptic character, physical characterization, ICP-OES, SEM analysis all these shows the drug is safe.

Therapeutic dose of *naga chenduram* is 488mg two times a day. As per OECD guideline three dose levels were selected for the study. They are low dose (X), mid dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose (488mg/kg) and the body surface area of the rat (0.018). i.e X dose is 90mg/kg, 5X dose is 450mg /kg,10X dose 900mg/kg.

The routine Hematological & Biochemical parameters were observed. Histopathology studies of various organs show no cytological changes.

The pharmacological Activity i.e Styptic activity, Anti-inflammatory activity and Analgesic activity shows satisfied results. There is no toxicity on administration.

*Naga chenduram* seems to be safe on animal experimentation studies may be carried out further for clinical evaluation.

# *AIM AND OBJECTIVES*

# 2. AIM AND OBJECTIVES

**Aim:**

To evaluate the Safety and pharmacological profile of the test drug ***"NAGA CHENDURAM''*** in animal models.

**Objectives:**

- Review of various information (Siddha and modern) relevant to the study.
- Preparation of the drug as per classical Siddha literature.
- Physicochemical, Chemical, elemental and analysis of the particle size of test drug.

**Toxicity studies:**

- Acute oral toxicity study (OECD – 423 Guideline)
- Repeated dose 28 days oral toxicity study (OECD – 407Guideline)
- Repeated dose 90 days oral toxicity study (OECD – 408Guideline)

**Pharmacological activities on wistar albino rats & Swiss albino mice:**

- Styptic activity (Tail cutting method)
- Anti-inflammatory activity (Cotton pellet induced granuloma method)
- Analgesic activity ( Eddy's hot plate method)

# *MATERIALS AND METHODS*

# 3. MATERIALS AND METHODS

**Drug selection:**

**"*NAGA CHENDURAM*"** was taken as a trial drug from the Siddha literature **"*AATHMA RAHSHAMIRTHAM ENNUM VAITHIYA SARA SANGIRAKAM*"** Page no-496, Edition-2006, Author - *Kandhasamy Muthaliyar.*

**Ingredients:**

| | |
|---|---|
| **1. Purified Nagam (**zinc**)** | **-** 4palam (140gm) |
| **2. Purified *Vediuppu (***Potassium nitrate*)* | - 1palam (35gm) |
| **3. Purified *Omam* (**carum copticum) | - 1/8*padi* (325gm) |
| **4. Purified *Chukku* (**zingiber officinale**)** | - 1/8*padi* (325gm) |
| **5. Purified *Manjal* (**curcuma longa) | - 1/8*padi* (325gm) |
| **6. Purified *Nayuruvi* (**Achyranthus aspera**)** | **-** 1100gm |

**Collection of the Plant materials**

The *Achyranthus aspera* were freshly collected from in and around Nagapttinum, Tamilnadu.

Other raw drugs were procured from raw drug shop in Parrys, Chennai.

**Identification and Authentication of the drug**

All the plant materials were identified and authenticated by the Botanist, Department of Medicinal botany, National Institute of Siddha..

**Purification of the drugs**

All the drugs mentioned here were purified as per the Siddha literature.

**Purification of *Nagam*:**

The ghee of south Indian *Madhuga* (*Madhuga longifolia)*- iluppai ghee is taken in a mud pot two pieces of ammonium chloride are placed in the pot in such a way that half of the portion of the pieces is immersed in the ghee on opposite direction. The zinc melted in an iron pots is poured twenty one times to the ghee of south Indian madhuga and purified zinc washed.

**Purification of vediuppu:**

Water is added to the salt and boiled on a hearth with mild flames. The white of eggs (4nos) is added to every 1400 gm of salt and the bubbles appearing with impure substances with a wooden spoon.

The ingredients are then transferred to another pot, sealed with mud pasted cloth, filtered and kept in places without aeration. Next day the water was filtered and salt was dried in sun shade. This process was repeated for seven times to got it purified.

**Purification of plant metrials:**

**Purification of *Chukku:***

*Chukku* was purified by removing the outer layer and soaking in the limestone water for three hours.

**Purification of *Omam:***

*Omam* was purified by soaking in the limestone water for three hours and then it was dried.

**Purification of *Manjal:***

*Manjal* was purified by removing the outer layer of skin.

**Purification on *Nayuruvi:***

Whole plant of *Achyranthus aspera* was cleaned well from dust and impurities and root only purified by running water.

**Standard operative Procedure:**

- *Nagam* was purified as mentioned by the Siddha text. The other ingredients were powdered and mixed well.
- The purified *Nagam* melted and it was subjected into *kirasam* process with the above powder, then it was ground with lemon juice and subjected in to *pudam* process.
- Finally chenduram were collected and stored in an air tight container

**Labeling:**

| | | |
|---|---|---|
| Name of the medicine | - | *NAGA CHENDURAM* |
| Date of preparation | - | 21.7.2015 |
| Dose | - | 488mg, Twice daily |
| Adjuvant/Vehicle | - | Honey |
| Indication | - | *Moolam, Kasam, Elai* |
| Date of expiry | - | 75 Yrs from the date of Preparation. |

**Therapeutic administration of drug**

| | | |
|---|---|---|
| Form of medicine | - | Chenduram |
| Route of administration | - | Oral |
| Dose | - | 488mg |
| Vehicle | - | Honey |
| Time of administration | - | Twice in a daily |

*Nagam* - Before Purification


*Nagam* –After Purification

*Vediuppu*- Before Purification

*Vediuppu*-After Purification

*Omam*-Before Purification

*Omam*-After Purification

*Chukku*-Before Purification

*Chukku*-After Purification

*Manjal*-Before Purification

*Manjal*-After Purification

*Nayuruvi*-Before Purication

*Nayuruvi*-After Purification

***Naga chenduram***

# *REVIEW OF LITERATURE*

# 4.1 **GUNAPADAM REVIEW**

## நாகம்

**வேறு பெயர்கள்:**

சீறல், பொருமல், பொங்கல், இரைச்சல், ஐம்புகை, சோரம், வாசுகி, தாம்பிரத்தின் வேதை, வாதத்திற்கு உயிர்.

**செய்கை**

குருதிப்பெருக்கடக்கி

உடல்தேற்றி

துவர்ப்பி

**குணம்**

மேகங் கிளர்பேதி வெட்டையழலை தணிக்கும்

வேகங் கிராணி விலக்குங்காண்-போகாப்

பரியமுளைப் புண்ணைப் பயித்தியத்தைப் போக்கும்

அரியதுத்த நாகமது.

(குணபாடம் தாதுசீவ வகுப்பு)

**பொருள்**

துத்தநாகத்தினால் மேகம், பேதி, உட்சுடு. கிரகணி, முளைப்புண், பித்தம் போகும்.

**நாகம் சேரும் மூல நோய்க்கான மருந்துகள்.**

1.மால்தேவி செந்தூரம்

2.முத்து செந்தூரம

3.நாகரச செந்தூரம்

# வெடியுப்பு

**வேறு பெயர்கள்:**

பொட்டிலுப்பு, இணங்கன், படைராசன், பூமி கூர்மை, நவச்சார மித்ரு,

**செய்கை:**

குளிர்ச்சி உண்டாக்கி

**பொதுகுணம்:**

சூதக வாயுவோடு சோணிதத்தின் வாதமும்போம்

வாதவலி குன்மமிவை மாறுங்காண் மீதாங்

கொடிய வயிறிழியுங் கோழைகப மேகும்

வெடியுப்புத் தன்னை விளம்பு. (குணபாடம் தாதுசீவ வகுப்பு)

**பொருள்**

வெடியுப்புனால் கீல்வாதம், இரத்த பித்தம், கண்ணோய், தொண்டை விரணம், சுவாசகாசம் நீங்கும்.

**மருத்துவ பயன்கள்**

- ஒரு பலம் வெடியுப்பை 2 ஆழாக்கு நீரிலிட்டு கலந்து சீலையை நனைத்து மூட்டு வீக்கம், மூட்டு வலி போட்டுவர குணமாகும்.
- நீர்க்கடுப்பு, தோல் வறட்சி, அம்மையால் காணும் சுரம் இவைகளுக்கு பொட்டிலுப்பு 2 வாரகனெடை, கஞ்சியில் கலந்து தேன் அல்லது கற்கண்டு சேர்த்து அருந்தலாம்.

**வெடியுப்பு சேரும் மூல நோய்க்கான மருந்துகள்:**

1. சிந்து வல்லாதகி மெழுகு

# ஓமம்

**வேறு பெயர்கள்:**

அசமோதம், தீப்பியம்

**பயன்படும் உறுப்பு:**

விதை

சுவை - கார்ப்பு

தன்மை - வெப்பம்

பிரிவு - கார்ப்பு

**செய்கை**

அழுகலகற்றி

இசிவகற்றி

உரமாக்கி

**பொதுகுணம்**

சீதசுரங் காசஞ் செரியாமந் தம்பொருமல்

பேதியிரைச் சல்கடுப்பு பேராமம்-ஓதிருமல்

பல்லொடுபல் மூலம் பகமிவைநோ யென்செயுமோ

சொல்லொடுபோம் ஓமமெனச் சொல். (அகத்தியர் குணவாகடம்)

**பொருள்**

ஓமத்தினால் குய்யரோகம், இரைப்பு, நீங்கும்.

**மருத்துவ பயன்கள்**

- ஓமம் 252 கிராம், ஆடாதோடைச்சாறு, இஞ்சிச்சாறு, பழரசம், புதினாச்சாறு வகைக்கு 136 கிராம், இந்துப்பு 34 கிராம் சேர்த்து ஊறவைத்து உலர்த்தி கொடுக்க இருமல் சுவாசகாசம், அசீரணம் நீங்கும்.

**ஓமம் சேரும் மூல நோய்க்கான மருந்துகள்:**

1.பூரணாதி இளகம்

2.காபட இளகம்

# சுக்கு

**வேறு பெயர்கள்**

அருக்கன், கடுபத்திரம், சுண்டி, சொண்டி, சௌபன்னம், நவசுறு, விடமுடிய அமிர்தம்.

**பயன்படும் உறுப்பு**

கிழங்கு (உலர்ந்தது)

சுவை - கார்ப்பு

தன்மை - வெப்பம்

பிரிவு - கார்ப்பு

**செய்கை**

பசித்தீத்துாண்டி

அகட்டுவாய்வகற்றி

**குணம்**

சூலைமந்தம் நெஞ்சரிப்பு தோடமேப் பம்பழலை

மூலம் இரைப்பிருமல் மூக்குநீர்-வாலகப

தோடமதி சாரந் தொடர்வாத குன்மநீர்த்

தோடம்ஆ மம்போக்குஞ் சுக்கு. (அகத்தியர் குணவாகடம்)

**பொருள்**

சுக்கினால் கீழ்வாய் நோய், இரைப்பு, இருமல், பாண்டு, குன்மம், செரியாமை இவை போம்.

**மருத்துவ பயன்கள்**

- சுக்கை அரைத்து மூட்டு வீக்கங்களுக்கு பூச வீக்கம் நாளுக்கு நாள் குறையும்.
- சுக்கை வாயிலிட்டு மெல்ல பல்வலி போம்

**சுக்கு சேரும் மூல நோய்க்கான மருந்துகள்:**

1.மேகராசங்க எண்ணெய்

2.கதலிப்பூ இராசயணம்

3.கருங்கோழி சூரணம்

# மஞ்சள்

**வேறு பெயர்கள்**

கான்சனி, அரிசனம், பீதம்

**பயன்படும் உறுப்பு**

கிழங்கு

சுவை -கார்ப்பு

தன்மை -வெப்பம்

பிரிவு -கார்ப்பு

**செய்கை**

அகட்டுவாய்கற்றி

வெப்பமுண்டாக்கி

ஈரல்தேற்றி

**குணம்**

நீர்க்கடுப்பு காசமொடு நீடு விடசுரமுந்

தீர்க்கநமைச் சல்வெபபுஞ் சேர்மலமும்- பார்க்குணமிக

அஞ்சியே யேகுமஞ்ச ளாம்வத்தி ரம்புனைந்தால்

வஞ்சியே! நன்றாய் வழத்து.

(அகத்தியர் குணவாகடம்)

**பொருள்**

மஞ்சளினால் ஐவகை வலி, வீக்கம், வண்டுகடி, பெரும்புண், தலைவலி, வளி, அழல் போகும்.

**மருத்துவ பயன்கள்**

- மஞ்சள் நீரை அருந்த காமாலை போம்.
- பச்சை மஞ்சள் இரசத்தைப் பூச புதிய காயப்புண், புண், வீக்கம் போம்

**மஞ்சள் சேரும் மூல நோய்க்கான மருந்துகள்:**

1.இலவங்காதி சூரணம்

# நாயுருவி

**வேறு பெயர்கள்**

அபமார்க்கி, காஞ்சரி, சனம், கதிரி, கிருஷ்ணபன்னி, மாமுனி, சேகரி, அபாமார்க்கம்.

**பயன்படும் உறுப்பு:**

செடி

சுவை - கைப்பு, துவர்ப்பு, கார்ப்பு

தன்மை - வெப்பம்

பிரிவு - கார்ப்பு

**செய்கை**

துவர்ப்பி

உடற்தேற்றி

**குணம்**

மலிகாரங் கைப்புள்ள அபமார்க்கி யின்வேரால் வசியமுண்டாம்

இலைமூல உதிரமந்தம் பேதிகபம் வியர்வுதந்தி யிறங்கு மேகம்

மலையேறும் படிபுரியு முள்ளரிசி பசிமாற்றும் வனச மூலம்

பலமாதர்க் குள்ளழுக்கை நீக்குவங்கச் சிந்தூரம் பண்ணுமாதோ

(அகத்தியர் குணவாகடம்)

**பொருள்**

இலை- கீழ்வாய் குருதி போக்கும், கழிச்சல், ஐயநோய், வியர்வை, வெள்ளை போக்கும்.

**மருத்துவ பயன்கள்**

- நாயுருவி விதையை அரிசி கழுவிய நீருடன் உட்கொண்டு வந்தால் மூலம் நீங்கும்.
- இலைச் சாற்றை வெயிலில் வைத்து வற்றச் செய்து மெழுகு போலாக்கி அத்துடன் பூண்டு சேர்த்துரைத்து கொறுக்கு புண்ணிற் போடப் போம்.

**நாயுருவி சேரும் மூல நோய்க்கான மருந்துகள்:**

1.நாயுருவி நெய்

# *ANALYTICAL STUDY OF TRIAL DRUG*

# 5. ANALYTICAL STUDY OF *NAGA CHENDURAM*

Analytical study of the drug brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Analytical study includes many studies such as its organoleptic properties, physical properties and also to assess the active principles and elements present in the drug. Thus analytical brings the efficacy and potency of the drug.

Analytical of the drug includes:

- **Organoleptic characters**
- **Physicochemical analysis**
    - Determination of Ash Values
    - Physical characterization
- **Chemical analysis**
    - Preliminary Basic and Acidic radical studies
- **Elemental analysis**
    - **ICP-OES**
- **Analysis of particle size**
    - **SEM**

## 5.1 Organoleptic characterization of *Naga chenduram*

### Colour

The medicine was taken in to watch glasses and placed against white back ground in white tube light. It was observed for its color by naked eye.

### Odour

The medicine was smelled individually. The time interval among two smelling was kept 2 minutes to nullify the effect of previous smelling. The results were showed in table 1.

**Physicochemical Analysis**

**5.2 Physical properties of *Naga chenduram***

The physical properties of *Naga chenduram* was analyzed at Captain Srinivasa murti Reseach Institute of Ayurveda and Siddha Drug Development, Arumbakkam, Chennai-106.

**Determination of Ash Values:**

**Total Ash**

3gm is accurately weighed and incinerated in a crucible dish at a temperature not exceed 450$^{o}$C until free from carbon. It is then cooled and weighed. The total ash % w/w with reference to the air-dried powder is calculated. The results were showed in table-2

**Water Soluble Ash**

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5minutes with 25ml water. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450$^{o}$C. The silica crucible and residue are weighed until constant weight is attained for determination of weight of insoluble ash. The weight of the water soluble ash is determined by subtracting the weight of insoluble ash from the weight of total ash.

**Acid insoluble Ash**

The total ash is obtained as the above method for preparation of total ash. The ash is boiled for 5minutes with 25ml 10% Hcl. The insoluble ashes is collected using filter paper and washed with hot water and then transferred to the silica crucible then ignite for 15minutes at temperature not exceeding 450$^{o}$C. The silica crucible and residue are weighed until constant weight is attained. The results were showed in table-3

**Loss on Drying**

The drug *Naga chenduram* was dried in the oven at 100- 105°C to constant weight. The results were showed in table 4.

## 5.3 CHEMICAL ANALYSIS OF *NAGA CHENDURAM*

The chemical analysis of naga chenduram was carried out in Bio chemistry Lab, national instutite of siddha. The results were showed in table 5,5a.

| S.No | EXPERIMENT | OBSERVATION | INFERENCE |
|---|---|---|---|
| 1. | Physical Appearance of extract | Red in colour | |
| 2. | **Test for Silicate**<br>a. A 500mg of the sample was shaken well with distilled water. | Sparingly soluble | Absence of Silicate |
| 3. | **Action of Heat:**<br>A 500mg of the sample was taken in a dry test tube and heated gently at first and then strong. | No White fumes evolved.<br>No brown fumes evolved. | Absence of Carbonate<br>Absence of Nitrate. |
| 4. | **Flame Test:**<br>A 500mg of the sample was made into a paste with con. HCl in a watch glass and introduced into non-luminous part of the Bunsen flame. | No bluish green flame | Absence of copper |
| 5. | **Ash Test:**<br>A filter paper was soaked into a mixture of extract and dil. cobalt nitrate solution and introduced into the Bunsen flame and ignited. | Appearance of yellow colour flame | **Presenceof sodium** |

**Preparation of Extract:**

5gm of sample was taken in a 250ml clean beaker and added with 50ml of distilled water. Then it is boiled well for about 10 minutes. Then it is cooled and filtered in a 100ml volumetric flask and made up to 100ml with distilled water. This preparation is used for the qualitative analysis of acidic/basic radicals and biochemical constituents in it.

| S.N | EXPERIMENT | OBSERVATION | INFERENCE |
|---|---|---|---|
| | **I. Test For Acid Radicals** | | |
| 1. | **Test For Sulphate:**<br>2ml of the above prepared extract was taken in a test tube to this added 2ml of 4% dil ammonium oxalate solution. | No cloudy appearance | Absence of Sulphate |
| 2. | **Test For Chloride:**<br>2ml of the above prepared extracts was added with 2ml of dil-HCl is added until the effervescence ceases off. | Cloudy appearance present. | **Presence of Chloride** |
| 3. | Test For Phosphate:<br>2ml of the extract were treated with 2ml of dil.ammonium molybdate solution and 2ml of con.HNo3 | No Cloudy yellow appearance present | Absence of Phosphate |
| 4. | **Test For Carbonate:**<br>2ml of the extract was treated with 2ml dil. magnesium sulphate solution | No cloudy appearance. | Absence of carbonate |
| 5. | **Test For Nitrate:**<br>1gm of the extract was heated with copper turning and concentrated $H_2So_4$ and viewed the test tube vertically down. | No Brown gas is evolved | Absence of nitrate |
| 6. | **Test For Sulphide:**<br>1gm of the extract was treated with 2ml of con. HCL | No rotten egg smelling gas is evolved | Absence of sulphide |

| | | | |
|---|---|---|---|
| 7. | **Test For Fluoride & Oxalate:**<br>2ml of extract was added with 2ml of dil. Acetic acid and 2ml dil.calcium chloride solution and heated**.** | No cloudy appearance. | Absence of fluoride and oxalate |
| 8. | **Test For Nitrite:**<br>3drops of the extract was placed on a filter paper, on that-2 drops of dil.acetic acid and 2 drops of dil.Benzidine solution is placed. | No characteristi c changes | Absence of nitrite |
| 9. | **Test For Borate:**<br>2 Pinches (50mg) of the extract was made into paste by using dil.sulphuric acid and alcohol (95%) and introduced into the blue flame. | No ppearance of bluish green colour | Absenceof borate |
| **II. Test For Basic Radicals** | | | |
| 1. | **Test For Lead:**<br>2ml of the extract was added with 2ml of dil.potassium iodine solution. | No Yellow precipitate is obtained | Absence of lead |
| 2. | **Test For Copper:**<br>**a.** One pinch (25mg) of extract was made into paste with con. HClin a watch glass and introduced into the non-luminuous part of the flame. | No blue colour precipitate | Absence of copper |
| 3. | **Test For Aluminium:**<br>To the 2ml of extract dil.sodium hydroxide was added in 5 drops to excess. | Shows characterist ic changes | Absence of Aluminium. |
| 4. | **Test For Iron:**<br>a. To the 2ml of extract add 2ml of dil.ammonium solution<br>b. To the 2ml of extract 2ml thiocyanate solution and 2ml of con HNo3 is added | Red colour appeared | **Presence of Iron** |

| | | | |
|---|---|---|---|
| 5. | **Test For Zinc:**<br>To 2ml of the extract dil.sodium hydroxide solution was added in 5 drops to excess and dil.ammonium chloride is added. | NoWhite precipitate is formed | Absence of Zinc |
| 6. | **Test For Calcium:**<br>2ml of the extract was added with 2ml of 4% dil.ammonium oxalate solution | Cloudy appearance and white precipitate is formed | **Pressence of calcium** |
| 7. | **Test For Magnesium:**<br>To 2ml of extract dil.sodium hydroxide solution was added in drops to excess. | White precipitate is obtained | **Presence of magnesium** |
| 8. | **Test For Ammonium:**<br>To 2ml of extract 1 ml of Nessler's reagent and excess of dil.sodium hydroxide solution are added. | No Brown colour appeared | Absence of ammonium |
| 9 | **Test For Potassium:**<br>A pinch (25mg) of extract was treated of with 2ml of dil.sodium nitrite solution and then treated with 2ml of dil.cobalt nitrate in 30% dil.glacial acetic acid. | Yellow precipitate is obtained | **Presence of potassium** |
| 10. | **Test For Sodium**:<br>2 pinches (50mg) of the extract is made into paste by using HCl and introduced into the blue flame of Bunsen burner. | No yellow colour flame evolved. | **Presence of sodium** |
| 11. | **Test For Mercury**:<br>2ml of the extract was treated with 2ml of dil.sodium hydroxide solution. | No Yellow precipitate is obtained | Absence of Mercury |
| 12. | **Test For Arsenic:**<br>2ml of the extract was treated with 2ml of dil.sodium hydroxide solution. | No Brownish red precipitate is obtained | Absence of arsenic |

| | | | |
|---|---|---|---|
| **III. Miscellaneous** | | | |
| 1. | **Test For Starch:**<br>2ml of extract was treated with weak dil.Iodine solution | No Blue colour developed | Absence of starch |
| 2. | **Test For Reducing Sugar:**<br>5ml of Benedict's qualitative solution was taken in a test tube and allowed to boil for 2 minutes and added 8 to 10 drops of the extract and again boil it for 2 minutes. The colour | No Brick red colour is developed | Absence of reducing sugar |
| 3. | **Test For The Alkaloids**:<br>a) 2ml of the extract was treated with 2ml of dil. potassium lodide solution.<br>b) 2ml of the extract was treated with 2ml of dil.picric acid.<br>c) 2ml of the extract was treated with 2ml of dil.phosphotungstic acid. | Yellow colour developed | **Presence of Alkaloid** |
| 4. | **Test For Tannic Acid:**<br>2ml of extract was treated with 2ml of dil.ferric chloride solution | No Blue-black precipitate is obtained | Absence of Tannic acid |
| 5. | **Test For Unsaturated Compound:**<br>To the 2ml of extract 2ml of dil.Potassium permanganate solution is added. | Potassium permanganate is not decolourised | Absence of unsaturated compound |
| 6. | **Test For Amino Acid:**<br>2 drops of the extract was placed on a filter paper and dried well. 20ml of Burette reagent is added**.** | No violet colour | Absence of amino acid |

| | | | |
|---|---|---|---|
| 7. | **Test For Type Of Compound:**<br><br>2ml of the extract was treated with 2 ml of dil.ferric chloride solution. | No green and red colour<br><br>No Violet colour developed<br><br>No Blue colour developed. | Absence of quinolepinephrine pyrocatechoantipyrineAliphatic amino acid and meconic acid.<br>Apomorphine salicylate and Resorcinol are absent<br><br>Morphine, Phenol cresol and hydrouinone are absent. |

## 5.4 ELEMENTAL ANALYSIS - (ICP-OES)

The analysis of heavy metals and trace elements were estimated by using inductively coupled plasma optical emission spectrometry (ICP-OES). The Experimental procedure was done at SAIF, IIT Madras, and Chennai-36.

**INDUCTIVELY COUPLED PLASMA OPTICAL EMISSIONS SPECTROMETRY**

**Introduction**

The element composition of a sample is often an important part of the information needed to assess its properties. Hence there is a need for scientific instrumentation like ICP-OES which plays a pivotal role in the determination of these elements. ICP-OES is widely employed for the estimation of metals and metalloids at trace, minor and major concentration.

**Principle**

In this technique, the high temperature plasma source atomizes the sample and excites the atoms resulting in emission of photons. The atoms of each element in the sample emit specific wavelength of light. The emission spectrum from the plasma is dispersed by an optical spectrometer, so that intension of the individual wavelength can be measured. The number of photons emitted is directly proportional to the concentration of the element. The photons may be detected either sequentially or simultaneously. Quantitative analysis is achieved by measuring the intensity of this specific wavelength and after performing the calibration using known standards.

Identifying the presence of emission at the wavelength characteristic of the element of interest obtaining quantitative information i.e., how much of an element is in sample can be accomplished using plots of emission intensity versus concentration called calibration curves.

Table ICP-OES operating conditions

| | |
|---|---|
| Rf frequency | 40M Hz |
| Range | 165-782 nm |
| Detection | Up to ppm level using SCD detector |

Perkin Elmer Optima 5300 DV was used for standard ICP-OES analysis

**Sample preparation – Microwave Digestion**

- Weight 0.25 g of test sample and transfer into a liner provided with instrument.
- Slowly add 9ml of Nitric acid or sulphuric acid such that no piece of sample sticks on the slide.
- Mix throughly and allow reacting for few minutes.
- Place the liner in the vessel jacket.
- Close the screw cap hand- tight in clockwise direction.
- Seal the vessel and placed in the rotor fixed in microwave.
- Set temperature to 180°C for 5 minutes, hold at 180°C for least 10 minutes.

  Allow the vessels to cool down to a vessel interior temperature below 60°C and to a vessel surface temperature (IR) below 50°C before removing the rotor.
- The digested sample was made upto 100ml with Millipore water.
- If visible insoluble particles exist, solution could be filtered through whatmann filter paper.
- Transfer the digested solution into plastic containers and label them properly.

**Results**

The analytical results of heavy metals and trace elements in Naga chenduram using ICP-OES are showed in table 6.

## 5.5ANALYSIS OF PARTICLE SIZE

## SCANNED ELECTRON MICROSCOPY (SEM)

**Study done:**

The particle size of the Naga chenduram was determined using High resolution scanning electron microscopy (HR-SEM).The Experimental procedure was done at SAIF, IIT Madras, and Chennai-36.

**Experimental procedure**:

A SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The primary electron beam interacts with the sample in a number of key ways:-

- Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen.
- Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast.
- Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-ray emitted are characteristic of the elements in the top few μm of the sample.

The SEM is carried out by using FEI Quanta FEG 200-High Resolution Instrument.

**Resolution:** 1.2nm gold particle separation on a carbon substrate.

**Magnification**: From a min of 12 x to greater than 1, 00,000 X.

**Application**: To evaluate grain size, particle size distributions, material homogeneity and inter metallic distributions.

# *TOXICITY STUDIES*

# 6. TOXICOLOGICAL EVALUATION OF *NAGA CHENDURAM* ON WISTAR ALBINO RATS

**Introduction**

Safety is a fundamental principle in the provision of traditional medicines and herbal products for health care and a critical

Component of quality control. OECD guidelines provide practical and technical guidance for monitoring the safety of traditional medicines within Pharmacovigilance systems. The safety monitoring of traditional medicines is compared and contrasted with that of other medicines, currently undertaken in the context of the WHO International Drug perspective.

**Scope of work:**

Monitoring Programme While there are regulatory and cultural differences in the preparation and use of different types of medicines, they are all equally important from a pharmacovigilance.

Assurance of safety, quality and efficacy of Indian System of Medicines (ISM) is the key issue that needs to be addressed while conducting toxicity studies. It is an essential step, which will strengthen the acceptance of Siddha medicines by scientific community. Information of toxicity and adverse effects of these formulations are lacking. Some of the formulations are proved to be effective in various animal studies and many more are yet to be tested.

Hence, the present study was carried out to evaluate the Preclinical animal toxicity studies of ***NAGA CHENDURAM*** in rodents.

The following studies were carried out on *NAGA CHENDURAM*

- Acute Oral toxicity – OECD 423
- 28-Days Repeated dose Oral Toxicity Study – OECD 407
- 90 days Repeated dose oral Toxicity study-OECD 408

# ACUTE AND SUBACUTE TOXICITY STUDIES OF "*NAGA CHENDURAM*'' ON WISTAR ALBINO RATS.

**Aim**:

To evaluate of the acute and sub acute toxicities of siddha drug "*Naga Chenduram*" on wistar albino rats.

**Test drugs**

The drug *Naga chenduram* which was prepared by the method described in standard text books of siddha medicine.

**Drug and preparation of stock solution**

The aqueous suspension of Naga *Chenduram* was prepared in 1% carboxymethylcellulose (CMC) solution in distilled water prior to oral administration to animals. It was used within seven days and stored at 8□C .While for futher use freshly prepared solution was used. The vehicle alone served as control.

**Experimental animals:**

Albino rats (wistar rats) of either sex, weighing (100-250 g) were procured from animal housing facility, K.k College of pharmacy, Gerugambakkam, Chennai. All animals were placed in a polypropylene cages in a controlled room temperature 24□ C±1□ C and relative humidity of 60-70 % in animal house. The animals were maintained in standard pellet diet and water ad libitum. They were acclimatized to laboratory condition for seven days before commencement of the experiment.

All the protocols and the experiments conducted in strict compliance according to ethical principles and guidelines provided by committee for the purpose of control and Supervision of Experiments on Animals. Animal experimentation protocols are approved by Institutional Animal Ethical Committee **(KKCP/2015/032)**.

### 6.1 Acute toxicity study (OECD – 423 Guideline)

The Acute toxicity studies were performed in accordance with the OECD 423 guidelines. Female wistar rats weighing 150-200gm were selected and divided into 4 groups containing three animals in a each group. The single dose of *Naga Chenduram* the dose starting from 5mg/kg, 50mg/kg, 300mg/kg and 2000mg/kg of body weight (5, 50,300, 2000mg/kg) was administered orally. The drug treated animals were carefully observed individually for the toxicity signs and mortality. The parameters such as changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous system, behavioural pattern, convulsions, tremors, salivation, lethargy, diarrhoea, sleep and coma were observed. From the maximum dose 1\5th or 1\10th of the dose was considered as therapeutic dose for further study. The results were showed in table -7.

## 6.2 28 days repeated dose oral toxicity study of "*Naga Chenduram*" on wistar albino rats (OECD – 407 guidelines)

Sub-acute toxicity studies were carried out according to OECD 407 and rats were divided into 3 groups of 10 animals (5 male and 5 female). *Naga Chenduram* was administered to rats at the dose of 450mg/kg/day and 900mg/kg/day for 28 days. The animals were observed daily for gross behavioral changes and other sign of sub acute toxicity. The weight of the each rat was recorded on day 0 and weekly throughout the course of the study, food and water consumption per rat was calculated. At the end of 28 days they were fasted overnight, each animal were anaesthetized with diethyl ether and blood samples were collected from the retro-orbital plexus into two tubes: one with EDTA for immediate analysis of hematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4°C for 10 minutes to obtain the serum. Serum was stored at 20°C until analyzed for biochemical parameters.

| | | |
|---|---|---|
| Test Substance | : | *Naga Chenduram* |
| Animal Source | : | Animal house of King Institute of Preventive Medicine |
| Animals | : | Male and Female Wistar Albino Rats |
| Age | : | More than 8 weeks |
| Acclimatization | : | Seven days prior to dosing. |
| Veterinary examination | : | Prior to at the end of the acclimatization period. |
| Identification of animals | : | By cage number, animal number and individual marking on fur. |
| Diet | : | Pelleted feed supplied by Godrej foods Pvt Ltd, Bangalore |
| Water | : | Potable water in polypropylene bottles *ad libitum.* |

| | | |
|---|---|---|
| Housing & Environment | : | The animals were housed in Polypropylene cages provided<br>With bedding of husk. |
| Housing temperature | : | Between 20 & 24°C, |
| Relative humidity | : | Between 30% and 70%, |
| Dark and light cycle | : | Each of 12 hours. |

**Justification for Dose Selection:**

The results of acute toxicity studies in rats indicated that *Naga Chenduram* was non toxic and no behavioral changes was observed up to the dose level of 2000mg/kg body weight. From the maximum dose 1\5th or 1\10th of the dose was considered as therapeutic dose for further study.The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

As per OECD guideline two dose levels were selected for the study. They are mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose **(488 mg) and the body surface area of the rat (0.018). i.e 5X dose is 450 mg/kg, 10X dose is 900mg/kg.**

**Preparation and administration of dose:**

*Naga Chenduram* two doses level 450 mg/kg and 900 mg/kg respectively were prepared. The test substance was prepared every 7 days once for 28 days. The control animals were administered vehicle only. Administration was by oral (gavage), once daily for 28 consecutive days.

## METHODOLOGY

### Randomization, Numbering and Grouping of Animals:

The rats randomly divided in to three groups in each group contain 10 animals (5M/5F) Animals acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was fur marked with picric acid. The females were nulliporous and non-pregnant.

### OBSERVATIONS:

Experimental animals were kept under observation throughout the course of study for the following:

### i) Body Weight:

Weight of each rat was recorded on day 0 and weekly intervals throughout the course of study and at termination to calculate relative organ weights. From the data, group mean body weights and percentage of body weight gain were calculated-Table - 8.

### ii) Food and water Consumption:

The quantity of food consumed by groups consisting of ten animals of for different doses was recorded at weekly interval. Food consumed per animal was calculated for control and the treated dose groups-Table 9 and 10

### iii) Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any were recorded.

### iv) Mortality:

All animals were observed twice daily for mortality during entire course of study.

**V) Laboratory investigation:**

Following laboratory investigations were carried out on day 29 in animals fasted over-night. On 29th day, the animals were fasted for approximately 18 h, then anesthetized with ether and blood samples were collected from the abdominal aorta into two tubes: one with EDTA for immediate analysis of hematological parameters, the other without any anticoagulant and was centrifuged at 4000 rpm at 4 °C for 10 minutes to obtain the serum. Serum was stored at 20 °C until analyzed for biochemical parameters.

**Hematological Investigations:**

Blood samples of control and experimental rats was analyzed for Hemoglobin content, Total red blood corpuscles (RBC), White blood corpuscles (WBC) count, Platelet, Mean corpuscular volume (MCV) and Packed cell volume (PCV). From the estimated values of RBC count (millions/mm3) and PCV (volumes percent), Mean corpuscular volume (MCV) was calculated. The results were showed in table -11

**Biochemical Investigations:**

Serum and Urine was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for Protein, Bilirubin, Urea, Uric acid, Creatinine, Triglyceride, Cholesterol and Glucose levels by using standard methods. Activities of Glutamate Oxaloacetate Transaminase/ Aspartate Aminotransferase (GOT/AST), Glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and Alkaline phosphatase were estimated as per the colorimetric procedure. The results were showed in table-12,13,14.

**Necropsy:**

All the animals were sacrificed on day 29. Necropsy of all animals was carried out and the weights of the organs including liver, kidneys, lungs, spleen, brain, heart, uterus and testis/ovaries were recorded. The relative organ weight of each animal was then calculated as follows; The results were showed in table 16.

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rats on sacrifice day (g)}} \times 100$$

**Histopathology:**

Histopathological investigation of the vital organs was done. The organ pieces (3-5µm thick) of the highest dose level of 900mg /kg were preserved and were fixed in 10% formalin and washed in running water . Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

The organs included Heart, Kidneys, Liver, spleen and Brain, Bone of the animals were preserved they were subjected to histopathological examination.

**Statistical analysis:**

Findings such as clinical signs of intoxication, body weight changes, food, water consumption, and hematology and blood chemistry were subjected to One-way Anova Followed by Dunnet's test using a computer software programme. (Graph Pad)

## 6.3 90-DAYS REPEATED DOSE ORAL TOXICITY STUDY OF *NAGA CHENDURAM* (OECD GUIDELINE - 408)

| | |
|---|---|
| **Test Substance** | : ***NAGA CHENDURAM*** |
| **IAEC APPROVAL NO** | **: NIS/2016/06** |
| **Animal Source** | : TheKing Institute of Preventive Medicine, Guindy, Ch-32. |
| **Animals** | : Wister Albino Rats (Male -12, and Female-12) |
| **Age** | **:** 6-8 weeks |
| **Body Weight** | **:** 150-200gm. |
| **Acclimatization** | **:** Seven days prior to dosing. |
| **Veterinary examination** | **:** Prior and at the end of the acclimatization period. |
| **Identification of animals** | **:** By cage number, animal number and individual marking by using Picric acid. |
| **Diet** | **:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore |
| **Water** | **:** Aqua guard portable water in polypropylene bottles. |
| **Housing & Environment** | **:** The animals were housed in Polypropylene cages provided with bedding of husk. |
| **Housing temperature** | **:** between 22ºC $\pm$ 3ºC. |
| **Relative humidity** | **:** between 30% and 70%, |
| **Air changes** | **:** 10 to 15 per hour |
| **Dark and light cycle** | **:** 12**:**12 hours. |
| **Duration of the study** | **:** 90 Days. |

## METHODOLOGY

### Randomization, Numbering and Grouping of Animals:

24 Wistar Albino Rats (12M + 12F) were selected and divided into 4 groups. Each group consist of 6 animals (3 Male and Female 3).First group treated as a control and other three group were treated with test drug (low, mid, high) for 90 days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

### Justification for Dose Selection:

As per OECD guideline three dose levels were selected for the study. They are low dose (X), mid dose dose (5X), high dose (10X). X is calculated by multiplying the therapeutic dose **(488 mg) and the body surface area of the rat (0.018). i.e X dose is 90mg/kg, 5X dose is 450mg/kg,10X dose is 900mg/kg**

### Preparation and Administration of Dose:

*NAGA CHENDURAM* was suspended in Honey with distilled water to obtain concentrations of 200mg/ml. It was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 90 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 90 consecutive days.

### OBSERVATIONS:

**Experimental animals were kept under observation throughout the course of study for the following:**

- **Body Weight:**

  Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study.The resuls were showed in table -17.

- **Clinical signs**:

  All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

- **Mortality:**

  All animals were observed twice daily for mortality during entire course of study.

- **Laboratory Investigations:**

  Following laboratory investigations were carried out on day 91 in animals fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Hematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minute.

**Hematological Investigations:**

Hematological parameters were determined using Hematology analyzer. The results were showed in table-18.

- **Biochemical Investigations:**

Biochemical parameters were determined using auto-analyzer. The results were showed in table( 23-24)

- **Histopathology:**

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin.

- **Statistical analysis:**

Findings such as clinical signs of intoxication, body weight changes, food consumption, hematology and blood chemistry were subjected to One-way ANOVA followed by dunnet't'test using a computer software programme - INSTAT-V3 version.

# *PHARMACOLOGICAL STUDIES*

# 7. PHARMACOLOGICAL STUDY

## 7.1 Styptic activity of *Naga chenduram* on wistar albino rats by Tail cutting method.

**Aim:**

To evaluate the Styptic activity of *Naga Chenduram* by Tail cutting method

**Materials Used:**

All experimental procedures described were reviewed and approved by the Institutional Animal Ethical Committee of K.K. College of Pharmacy, Chennai-122, and IAEC approval **(KKCP/2015/032).**

**Experimental Animals:**

Wistar Albino rats of either sex, weighing 150 - 200 g were purchased from King Institute of Preventive medicine Animal House, Chennai, India. The animals were fed on standard rodent pellet and RO water was provided ad libitum. The animals were kept for overnight fasting before experimentation.

**Drug Treatment:**

Animals were randomized into three groups of six animals each.

Group I : Control (2% Carboxy Methyl Cellulose 2ml p.o

Group II : Naga chenduram drug at the dose of 200 mg/kg.

Group III : Naga chenduram drug at the dose of 400 mg/kg.

The animals were administered the drug orally and the blood sample were collected periodically for evaluation.

**Procedure:**

The animals were sacrificed after 24 hours of last dose of the test drug. The tail was cut with asalpel 1-2 cm proximal from the end, to study the bleeding time and clotting time. Subsequently blood was taken directly from the retro orbital vein in plain tube for studying the prothrombin time. The serum was separated by cold centrifuging machine at 4000 to 5000r.p.m for 15 minutes.

**Bleeding time method (BT)** :

Rat's tail was cut with a scapel 1-2 cm proximal from the end and bleeding time was calculated from the time of starting of bleeding till bleeding stopped. Spots were made with the bleeding tail on a blotting paper every 15 seconds till bleeding stopped and bleeding time was calculated accordingly.

**Clotting time(CT):**

Blood was drawn in to a capillary tube. The time of appearance of the drop of the blood on the cut tail was noted. The glass tube is the then kept between the palms of the both hands for 30 seconds to kept it at body temperature. After 30 seconds the tube was taken out and small portion of the capillary tube was broken at regular intervals of 10 seconds, until a thread of clotted blood appears between the two pieces of capillary glass tube. The time intervals between the appearance of the drop of the blood and the thread of the blood clot were the clotting time of rat expressed in minutes.

**Pro-thrombin time (PT):**

The prothrombin time was measured in terms of minutes taken by a sample of blood to form a clot in presence of Thromboplastin and calcium ions.

**Statistical analysis**

All experimental results were expressed as mean ± SEM statistics was determined by student t test followed by Dennett's T Test. By using Grap PAD Prism 5. The results were showed in table-25.

## 7.2 Anti-inflammatory activity of *Naga chenduram* on Wistar albino rats by cotton pellet induced granuloma method.

**Aim:**

To evaluate the anti-inflammotry activity of *Naga Chenduram* by cotton pellet induced granuloma method.

**Animals**

Animal : Albino Wistar rat

Sex : Male and female

Weight : 150-200 gm

Animals per Group : 6

Number of groups : 4

**Experimental Design for Cotton pellet granuloma model**

Group-I : control received -Honey (dose: 10 ml/kg).

Group-II : Animals treated with Dexamethasone (dose: 0.5 mg/kg).

Group-III : Animals treated with *Naga Chenduram* (dose: 200 mg/kg).

Group-IV : Animals treated with *Naga Chenduram* ( 400 mg/kg)

**Experimemental procedure:**

Inflammation was induced by cotton pellet granuloma model. This method was carried out by using sterilized cotton pellet implantation method in rats. Under light ether anesthesia by using blunted forceps and subcutaneous tunnel was made and sterilized cotton pellets (10 ± 1 mg) were implanted in the scapula of the rat. After recovering from Anaesthesia, animals were treated orally with vehicle control (Distilled water 10 ml / kg), Dexamethasone 0.5 mg/kg and low dose of (200mg/kg) and high dose(400mg/kg) of Naga chenduram for consecutive 7 days, once per day.

They were sacrificed on day 8th by cervical dislocation and the pellets were removed and immediate the wet weight was taken, freed from extraneous tissue and dried at $60^0$C for 24 hrs. The percentage inhibition of the wet weight and dry weight of the granuloma were calculated and compared.

$$\text{Percentage inhibition (\%)} = \frac{\text{Control - Treated}}{\text{Control}} \text{ X } 100$$

**Statistical analysis**

Results were expressed as mean ± SEM and analyzed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied. The results were showed in table 26.

## 7.3 Analgesic activity of *Naga chenduram* on Swiss albino mice by eddy's hot plate method.

**Aim:**

To evaluate the analgesic activity of *Naga Chenduram* by Eddy's hot plate method.

**Animals:**

Swiss albino mice of weighing (20-25gms) were used for this study and divided in to 4 groups. This each groups are animals 6(3M, 3F) .The animals were obtained from animal house, k.k college of pharmacy, Gerugambakkam, Chennai. Animals were housed at a temperature of 24±2$^{o}$c and relative humidity of 30-70%. At 12:12 light, day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pellet. All the experimental procedures and protocols used in this study were reviewed by (IAEC) Institutional Animal Ethics Committee **(KKCP/2015/032)** of k.k college of Pharmacy and were in accordance with the guidelines of the IAEC.

**Grouping:**

Group I - 2% distilled water (10ml/kg, p.o.),

Group II - Pentazocine (5mg/kg, p.o.)

Group III - *Naga Chenduram(*200mg/kg)

Group IV - *Naga Chenduram* (400mg/kg)

**Procedure:**

Animals were weighed and placed on the hot plate. Temperature of the hot plate was maintained at 55±1$^{o}$C. Responses such as jumping, withdrawal and licking of the paws were seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid

tissue damage of the animals 10 seconds was kept as a cut off time. The time obtained was considered the basal/normal reaction time in all the untreated groups of animals. Increase in the basal reaction time was the index of analgesia. All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. A final reading of the basal reaction time was recorded for the included animals. After selecting the animals, the drugs were administered to all the groups at the stipulated doses. The reaction times of the animals were then noted at 0, 30, 60, 90,120and 150 min interval after drug administration.

**Statistical analysis**

Results were expressed as mean ± SEM and analyzed using Graph Pad Prism software. One way analysis of variance (ANOVA) test was applied. The results were showed in table 27.

# *RESULTS*

## 8.RESULTS

**Table 1: Organoleptic characters of** *Naga* ***chenduram***

| S.no | Parameters | Results |
|---|---|---|
| 1 | Colour | Red |
| 2 | Odour | Characteristic odour |
| 3 | State of matter | Solid |
| 4 | Texture | Fine powder |

**Table 2: Physicochemical properties of *Naga chenduram***

**Determination of Ash Values: Percentage of Ash values**

| Parameter | Percentage% |
|---|---|
| Ash Value(%w/w) | 96.91% |

**Table 3**: Percentage of Acid insoluble ash values

| Parameter | Percentage% |
|---|---|
| Acid insoluble Ash(%w.w) | 5.43% |

**Interpretation for ash values**

**Ash**: Ash constitutes the inorganic residues obtained after complete combustion of a drug. Thus Ash value is a validity parameter describe and to assess the degree of purity of a given drug

**Total ash**: Total ash value of herbo mineral material indicated the amount of minerals and earthy materials present in the plant material. The total inorganic content (potassium, calcium, chloride, iron, etc.,) present in the drug is measured through the Total ash value and it is of **96.91 %** for *NAGA CHENDURAM*

**Acid insoluble ash**: The acid insoluble ash value of the drug denotes the amount of siliceous matter present in the plant. The quality of the drug is better if the acid insoluble value is low. It is **5.43%** for *NAGA CHENDURAM*

**Loss on Drying Table 4:** Percentage **Loss in weight on drying**

| **Parameter** | **Percentage%** |
|---|---|
| Loss on drying 105$^{o}$C | 0.53% |

**Interpretation**

- The total of volatile content and moisture present in the drug was established in loss on drying.
- Moisture content of the drug reveals the stability and its shelf-life.
- High moisture content can adversely affect the active ingredient of the drug. Thus low moisture content could get maximum stability and better shelf life.

**Table 5: Chemical Analysis of *Naga chenduram***

| **S.NO** | **Parameter** | **Results** |
|---|---|---|
| 1 | Slicate | Absent |
| 2 | Sulphate | Absent |
| 3 | Chloride | **Present** |
| 4 | Phosphate | Absent |
| 5 | Carbonate | Absent |
| 6 | Nitrate | Absent |
| 7 | Sulphate | Absent |
| 8 | Oxalate | Absent |
| 9 | Nitrate | Absent |
| 10 | Borate | Absent |
| 11 | Lead | Absent |
| 12 | Copper | Absent |
| 13 | Aluminium | Absent |

**Interpretation**: The acidic radicals test shows presence of chloride

**Table 5.a: chemical Analysis of *Naga chenduram***

| S.NO | Parameters | Results |
|---|---|---|
| **14.** | Iron | **Present** |
| **15.** | Zinc | Absent |
| **16.** | Calcium | **Present** |
| **17.** | Magnesium | **Present** |
| **18.** | Ammonium | Absent |
| **19.** | Potassium | **Present** |
| **20.** | Sodium | **Present** |
| **21.** | Mercury | Absent |
| **22.** | Arsenic | Absent |
| **23.** | Starch | Absent |
| **24.** | Reducing sugar | Absent |
| **25.** | Alkaloids | **Present** |
| **26.** | Tannic acid | Absent |

**Interpretation:**

The basic radicals test shows the presence of calcium, potassium, sodium, magnesium and absence of heavy metals such as lead, mercury, arsenic.

**ELEMENTAL ANALYSIS**

**INDUCTIVELY COUPLED PLASMA OPTICAL EMISSION SPECTORCOPY**

**Table 6: ICP-OES Study results of Naga chenduram**

| S.NO | Elements | Wavelength in nm | *Naga chenduram* mg/L |
|---|---|---|---|
| 1 | Arsenic | As 188.979 | BDL |
| 2 | Calcium | 315.807 | 12.990mg/L |
| 3 | Cadmium | 228.802 | BDL |
| 4 | Copper | 327.393 | BDL |
| 5 | Mercury | 253.652 | BDL |
| 6 | Potassium | 766.491 | 123.221mg/L |
| 7 | Sodium | 589.592 | 04.510mg/L |
| 8 | Nickel | 231.604 | BDL |
| 9 | Lead | 220.353 | BDL |
| 10 | Phosphorus | 213.617 | 05.321mg/L |
| 11 | Zinc | 206.200 | 124.288mg/L |

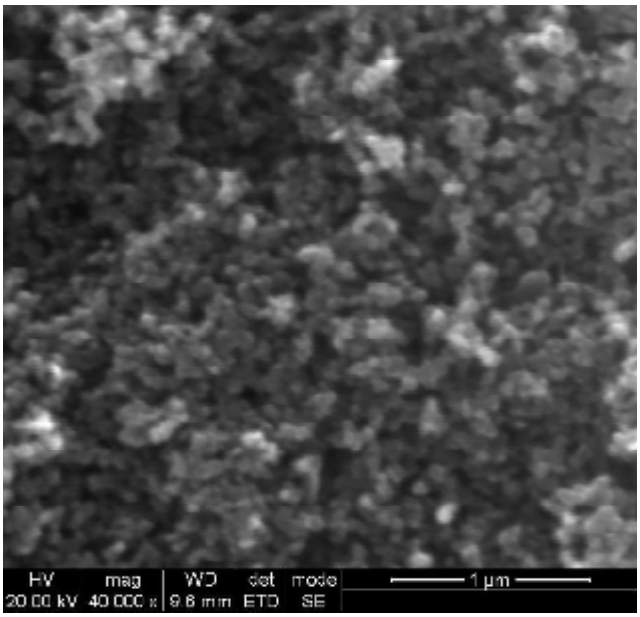
* BDL-Below Detection Limit

**Results:**

The results show the quantitative of the elements in *Naga chenduram*. The heavy metals were found to be within normal limits. The presence of other elements shows the therapeutic value of *Naga chenduram*. Hence the drug *Naga chenduram* is considered as a safe drug.

## ANALYSIS OF PARTICLE SIZE-SCANNED ELECTRON MICROSCOPY

**Determination of Particle size of *Naga chenduram***




**Results:**

**The picture shows that the particles are stabilize, have irregular morphology and distributed in the near nano range. *NAGA CHENDURAM* has particle size of 1to 2µm**

**Table7: Acute toxicity study of *Naga chenduram***

**Dose finding experiment and its behavioral Signs of acute oral toxicity**

| No | Dose mg/kg | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | + | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 2 | 50 | + | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | 300 | + | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | 2000 | + | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

1.Alertness 2.Aggressiveness 3.Pile erection 4.Grooming 5.Gripping 6.Touch Response 7.Decreased Motor activity 8.Tremors 9.Convulsions 10.Muscle Spasm 11.catatonia 12.Muscle relaxant 13.Hypnosis 14.Analgesia 15.Lacrimation 16.Exophthalmos 17.Diarrhoea 18. Writhing 19.Respiration 20.Mortality

+ Presence of activity

- Absence activity

**Results:**

All the data were summarized in the form of table (8) revealed no abnormal signs and behavioral changes in rats at the dose of 5, 50, 300, 2000mg/kg body wt administered orally.

**Gross necropsy:**

No abnormalities seen in external observation and examination on the dose level of 5, 50, 300, 2000mg/kg body weight. All vital organs were normal.

**28 days Repeated dose oral toxicity study of *Naga chenduram***

**Table 8:Body weight (g) of albino rats exposed to *Naga Chenduram* for 28 days**

| Dose (mg/kg/day) | Days | | | | |
|---|---|---|---|---|---|
| | 0 | 7th day | 14th day | 21th day | 28th day |
| Control | 128±11.22 | 129.83±10.52 | 130.83±9.89 | 132.83±15.37 | 131.33±13.95 |
| Mid dose | 134±17.10 | 135±14.83 | 135.16±12.1 | 137.5±10.3 | 140.66±7.69 |
| High dose | 136.5±13.11 | 141.5±11.65 | 141.33±11.2 | 141.5±7.99 | 141.66±9.99 |

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

**Table 9.Water (ml/day) intake of albino rats exposed to *Naga Chenduram* for 28 days**

| Dose(mg/kg/day) | Days(ml/rat) | | | | |
|---|---|---|---|---|---|
| | 0 | 7th day | 14th day | 21th day | 28th day |
| Control | 32.83±2.14 | 33.66±1.86 | 33.0±2.37 | 33.33±1.97 | 33.66±2.58 |
| Mid dose | 37.0±1.26 | 39.50±1.52 | 40.66±2.50 | 40.33±1.63 | 41.83±2.32 |
| High dose | 40.66±1.75 | 40.5±1.76 | 41.5±2.17 | 41.5±3.56 | 41.83±2.23 |

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

**Table10.Food (g/day) intake of albino rats exposed to *Naga Chenduram* for 28 days**

| Dose (mg/kg/day) | Days (gms/rats) | | | | |
|---|---|---|---|---|---|
| | 0 | 7th day | 14th day | 21th day | 28th day |
| Control | 36.11±1.12 | 35.70±2.21 | 37.43±1.25 | 39.28±1.88 | 39.7±2.29 |
| Mid dose | 38.15±1.71 | 39.83±2.14 | 40.51±37.26 | 37.26±5.04 | 36.93±4.00 |
| High dose | 40.32±2.02 | 39.22±1.43 | 41.78±1.09 | 41.10±1.91 | 42.15±1.37 |

Values are mean of a 10 animals ± S.E.M (Dunnet's test)* p<0.05;**p<0.01.N=10

**Table 11.Hematological parameters after 28days treatment with *Naga Chenduram***

**in rats**

| Parameters | Control | 450mg/kg | 900mg/kg |
|---|---|---|---|
| Red blood cell($mm^3$) | 7.78±0.56 | 8.18±0.59 | 8.70±0.62 |
| HB(%) | 14.36±0.29 | 14.33±1.03 | 14.86±0.43 |
| Leukocyte ($x10^6$/ml) | 1028±27.00 | 1014±44.50 | 1019±53.80 |
| Platelets/ul | 1325±86.43 | 1414±80.27 | 1433±37.79 |
| MCV(gl) | 51.25±0.88 | 52.96±1.36 | 53.71±0.92 |
| PCV(gl) | 37.6±0.50 | 37.5±0.35 | 37.6±0.26 |

Values are mean of a 10 animals ± S.E.M (Dunnet's test)* p<0.05;**p<0.01.N=10

**Chart 1:The mean value of HB, RBC, PCV and MCV of control and treated groups of wistar albino rats exposed to *NAGA CHENDURAM***

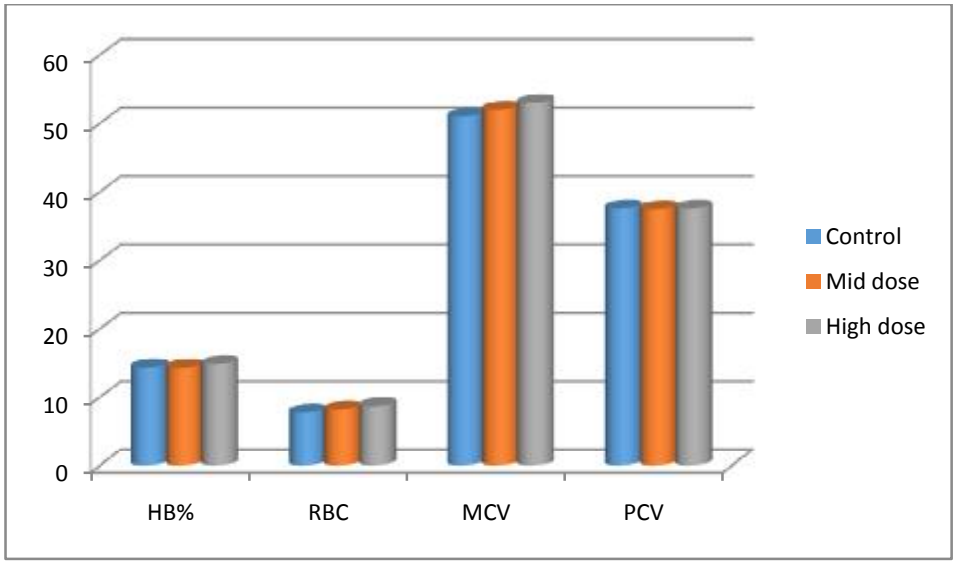


**Chart 2:The mean value of TWBC control and treated groups of wistar albino rats exposed to *NAGA CHENDURAM***

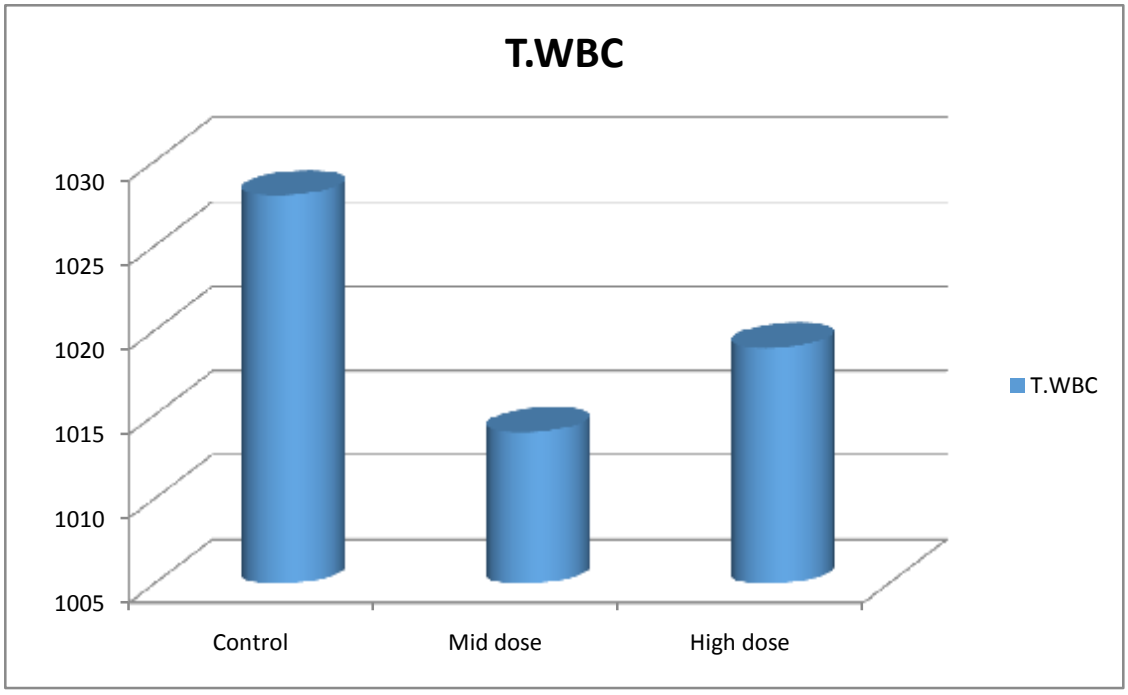



Chart 3:The mean value of **Platelet** control and treated groups of wistar albino rats exposed to *NAGA CHENDURAM*

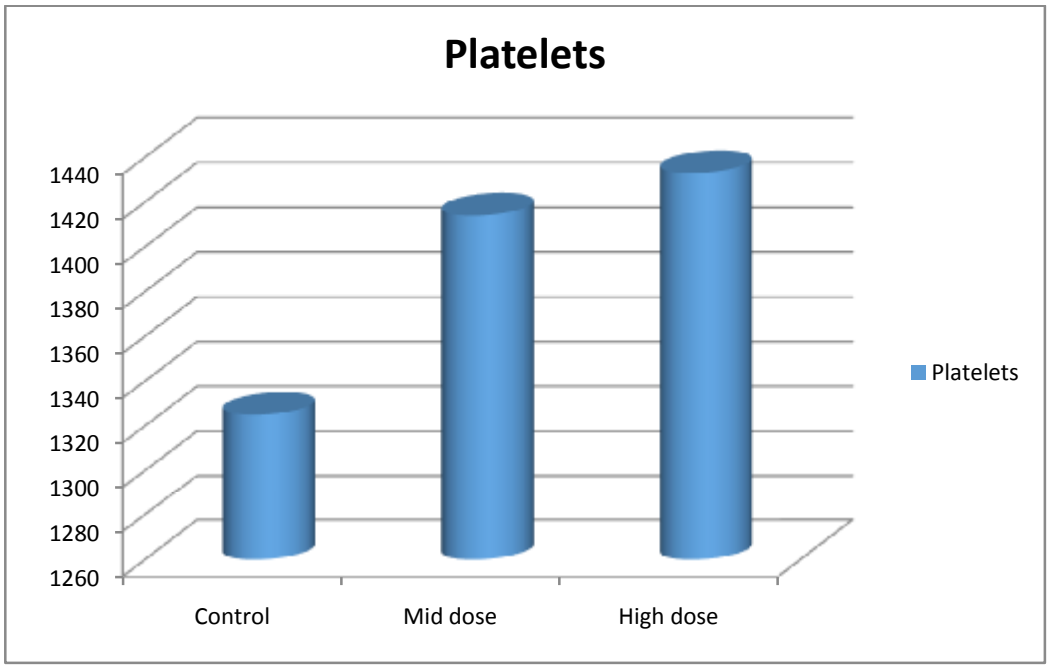


**Table 12: Sub acute toxicity -Liver function test of Wistar albino rats group exposed to Naga chenduram**

| Parameters | Control | Mid dose | High dose |
|---|---|---|---|
| Total Bilirubin(mg/dl) | 0.2012±0.01 | 0.2014±0.01 | 0.2014±0.01 |
| Bilirubin direct(mg/dl) | 0.1±0.02 | 0.1±0.02 | 0.1±0.02 |
| Bilirubin indirect(mg/dl) | 0.1±00 | 0.1±00 | 0.1±00 |
| ALP(U/L) | 372.3±3.27 | 365.16±4.12 | 352.66±3.20 |
| SGOT(U/L) | 159.83±2.04 | 152.16±2.40 | 150.5±1.52 |
| SGPT(U/L) | 38.06±1.21 | 40.85±1.05 | 42.91±1.61 |
| Total protein(g/dl) | 10.40±0.19 | 9.29±0.07 | 9.25±0.12 |
| Albumin(g/dl) | 3.04±0.04 | 3.04±0.03 | 3.06±0.04 |
| Globulin(g/dl) | 6.07±0.07 | 5.43±0.31 | 5.19±0.07 |

Values are mean of a 10 animals ± S.E.M (Dunnet's test)* p<0.05;**p<0.01.N=10

**Chart.4:The mean value of T.Bilirubin, D.Bilirubin,I.Bilirubin of control and treated groups of wistar albino rats exposed to *NAGA CHENDURAM***

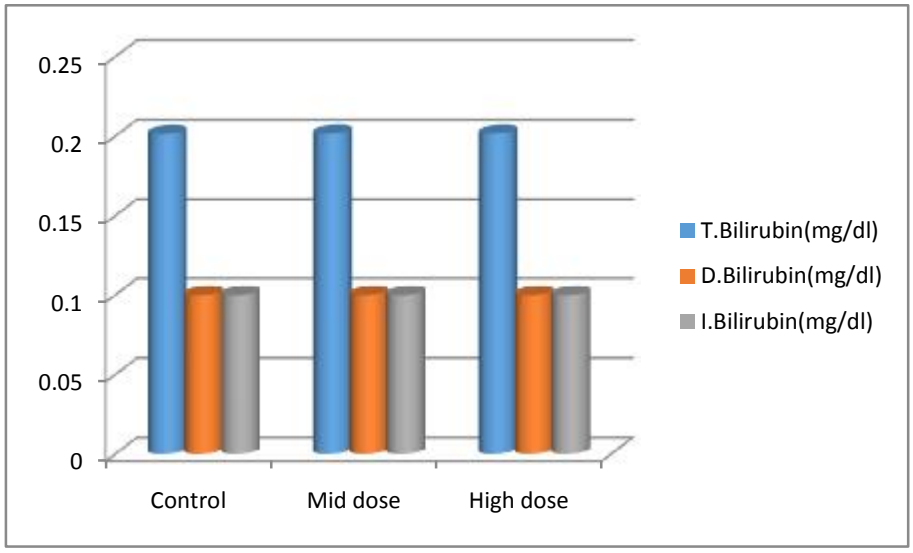


**Chart 5:The mean value of SGOT, SGPT, ALP of control and treated groups of wistar albino rats exposed to *NAGA CHENDURAM***

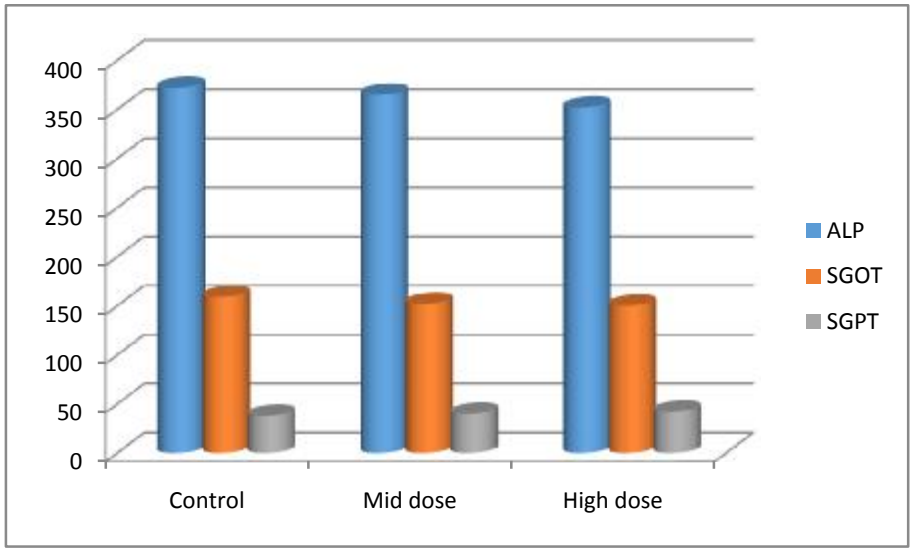



**Table 13: Sub acute toxicity –Renal function test of Wistar albino rats group exposed to Naga chenduram**

| Dose (mg/kg) | Control | Mid dose | High dose |
|---|---|---|---|
| **Urea(mg/dl)** | 53.29±0.94 | 52.41±0.94 | 52.05±1.39 |
| **Creatinine(mg/dl)** | 0.72±0.01 | 0.74±0.02 | 0.74±0.02 |
| **Uric acid(mg/dl)** | 1.4±0.02 | 1.4±0.02 | 1.4±0.04 |
| **Na m.mol** | 130.80±0.98 | 131.50±0.84 | 132.60±1.03 |
| **K m.mol** | 18.10±0.07 | 18.15±0.05 | 18.67±0.42 |
| **Cl m.mol** | 97.50±0.10 | 99.20±0.20 | 99.33±1.10 |

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

**Chart 6:The mean value of urea, creatinine, uric acid of control and treated groups of wistar albino rats exposed to *NAGA CHENDURAM***

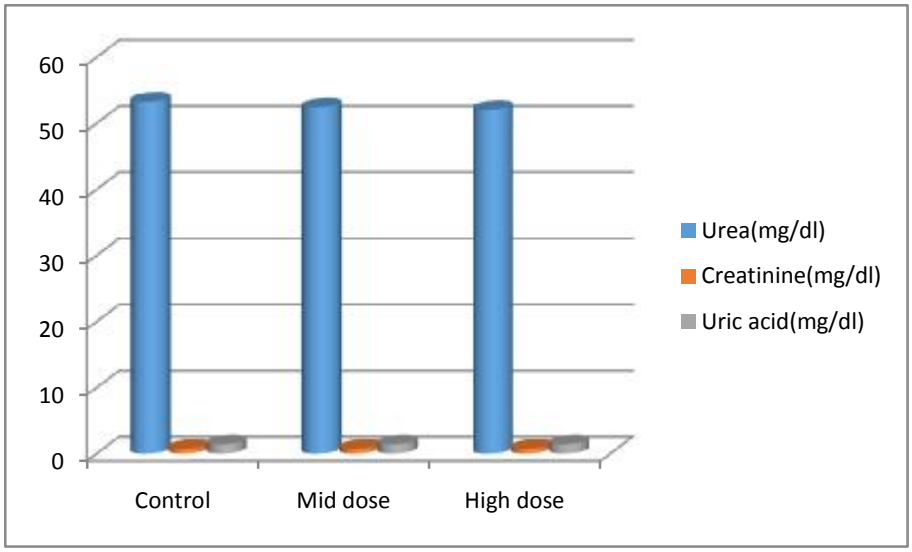



**Table 14: Sub acute toxicity – Lipid Profile of Wistar albino rats group exposed to Naga chenduram**

| Parameters | Control | Mid dose | High dose |
|---|---|---|---|
| **Total cholesterol (mg/kg)** | 34.89±0.50 | 34.68±0.51 | 35.18±.0.50 |
| **HDL(mg/dl)** | 12.07±0.41 | 12.32±0.18 | 12.15±0.07 |
| **LDL(mg/dl)** | 36.36±1.42 | 36.64±1.52 | 37.46±1.09 |
| **VLDL (mg/dl)** | 15.85±0.09 | 15.81±0.11 | 15.48±1.25 |
| **Triglycerides(mg/kg)** | 79.44±0.01 | 77.34±2.16 | 77.89 ±2.29 |
| **TC/HDL ratio (g/dl)** | 2.46±0.12 | 3.42±1.23 | 3.20±0.12 |
| **Blood glucose (mg/dl)** | 122.81±1.48 | 122.78±0.79 | 122.41±0.54 |

Values are mean of a 10animals ± S.E.M (Dunnet's test) *p<0.05 ;**p<0.01.N=10

**Chart 7:The mean value of CHOLESTROL, HDL,LDL ,TRIGLYCERIDES of control and treated groups of wistar albino rats exposed to *NAGA CHENDURAM***

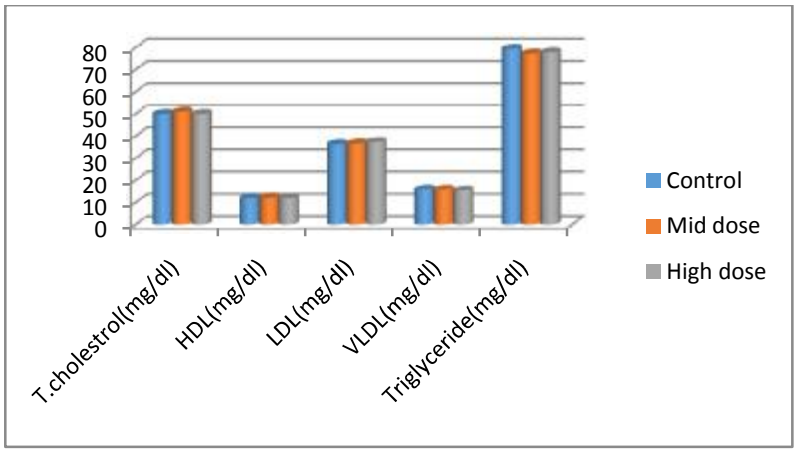



**Table 15: Sub acute toxicity – Urine Analysis of Wistar albino rats group exposed to Naga chenduram**

| **Parameters** | **Control** | **Mid dose** | **High dose** |
|---|---|---|---|
| **Transparency** | Clear | Slightly turbid | Slightly turbid |
| **Specific gravity** | 1.010 | 1.010 | 1.010 |
| **PH** | >7.0 | >7.2 | >7.2 |
| **Protein** | Nil | Nil | Nil |
| **Glucose** | Nil | Nil | Nil |
| **Bilirubin** | -ve | -ve | -ve |
| **Ketones** | -ve | -ve | -ve |
| **Blood** | Absent | Absent | Absent |
| **Urobilinogen** | Normal | Abnormal | Abnormal |
| **Pus cells** | 0-cells/HPF | 0-cells/HPF | 1-cells/HPF |
| **RBC** | Nil | Nil | 1-cells/HPF |
| **Epithelial cells** | Nil | 1-cells/HPF | Nil |
| **Crystals** | Nil | Nil | Nil |
| **Casts** | Nil | Nil | Nil |
| **Others** | Bacteria seen | Bacteria seen | Bacteria seen |
| **Colour** | Yellow | Yellow | Yellow |

**Table 16: Sub acute toxicity -Effect of oral administration of *Naga Chenduram* on organ weight**

| Dose (mg/kg) | Control | Mod dose | High dose |
|---|---|---|---|
| **Liver(g)** | 4.36±0.22 | 4.53±0.04 | 4.50±0.08 |
| **Heart (g)** | 0.39±0.04 | 0.39±0.03 | 0.39±0.02 |
| **Lung(g)** | 1.30±0.1 | 1.30±0.1 | 1.30±0.1 |
| **Spleen (g)** | 0.47±0.01 | 0.50±0.01 | 0.52±0.22 |
| **Ovary (g)** | 1.48±0.04 | 1.50±0.01 | 1.51±0.01 |
| **Testes(g)** | 1.20±0.22 | 1.22±0.22 | 1.22±0.28 |
| **Brain(g)** | 1.36±0.22 | 1.38±0.24 | 1.40±0.24 |
| **Kidney(g)** | 0.60±0.04 | 0.62±0.04 | 0.62±0.04 |
| **Stomach(g)** | 1.35±0.02 | 1.38±0.02 | 1.38±0.02 |

Values are mean of a 10 animals ± S.E.M (Dunnet's test) *p<0.05;**p<0.01.N=10

**Results:**

**Interpretation of Sub-acute toxicity of *Naga chenduram***

All animals from control and all the treated dose groups survived throughout the dosing period of 28 days for sub acute toxicity study. There was no significant change in the body weight for the control and treatment group throughout the dosing period of 28 days.

**Interpretation of hematological investigation**

The results of hematological investigations conducted on day 29$^{th}$ day revealed no significant changes in the hematological values when compared with those of respective controls. All the values were within the normal biological and laboratory limits.

**Interpretation of Biochemical investigation**

Results of Biochemical investigations conducted on days 29 and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls. All the values were within the normal biological and laboratory limits.

**Intrepretation of histopathology:**

The vital organs such as liver, heart, kidneys, bone, spleen and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Cross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control.

Organ weights of treated animals with respective control animals on day 29 were found to be comparable with respective control group. Gross pathological examination of animals did not reveal any abnormalities. Histopathology examination did not reveal any abnormal macroscopic changes.

**Table 17: Sub chronic toxicity study - Body weight of wistar albino rats group exposed to *NAGA CHENDURAM***

| DAYS | Weight(gms)/Days | | | | P value (p)* |
|---|---|---|---|---|---|
| | Control | Low dose | Mid dose | High dose | |
| 1 | 162.4±29.65 | 154.4 ± 21.83 | 155.6± 13.09 | 161.5± 28.17 | NS |
| 15 | 170.1 ± 28.49 | 162.8 ± 23.54 | 171.7 ±29.83 | 179.8 ± 32.13 | NS |
| 30 | 184.4 ± 28.83 | 184.71 ±14.76 | 182.8 ±32.17 | 190.2 ± 28.55 | NS |
| 45 | 202.7± 27.81 | 205.6± 29.12 | 203.1± 19.07 | 207.9± 22.05 | NS |
| 60 | 226.6±33.47 | 232.6±23.91 | 230.6±33.61 | 226.6±23.63 | NS |
| 75 | 240.8 ± 26.76 | 244.8 ± 28.08 | 242.0± 28.70 | 240.8 ±26.87 | NS |
| 90 | 263.5± 27.94 | 266.9± 27.68 | 268.2± 27.31 | 266.1± 29.41 | NS |

NS- Not Significant, ** ($p > 0.01$),*($p > 0.05$), n = 6 values are mean ± S.D (One way ANOVA followed by Dunett's test.

**Table 18. Hematological parameters after 90days treatment with *Naga Chenduram***

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| Redblood cell($mm^3$) | 7.33±0.175 | 7.45±0.327 | 7.4±0.303 | 7.7±0.189 |
| HB(%) | 12.8±0.35 | 12.93±0.5 | 12.95±0.73 | 13.18±0.68 |
| Leukocyte ($x10^6$/ml) | 10550±48 | 11060±541 | 11083±796 | 11200±258 |
| Platelets/ul | 3.45±0.28 | 3.4±0.14 | 3.51±0.46 | 3.91±0.14 |
| MCV(gl) | 89.16±2.71 | 90.33±3.07 | 90.66±2.33 | 91.83±4.02 |
| MCH(gl) | 32±4.0 | 30.66±1.86 | 32±2.1 | 31.83±0.75 |
| MCHC | 36.5±4.27 | 33.3±2.50 | 32.66±2.25 | 33±1.67 |

Values are mean of a 6 animals ± S.E.M (Dunnet's test)* $p<0.05$;**$p<0.01$.N=6

Chart 8: **Sub chronic toxicity - The mean value of HB, RBC, MCV, MCHC, MCH control and treated groups of wistar albino rats exposed to Naga chenduram**

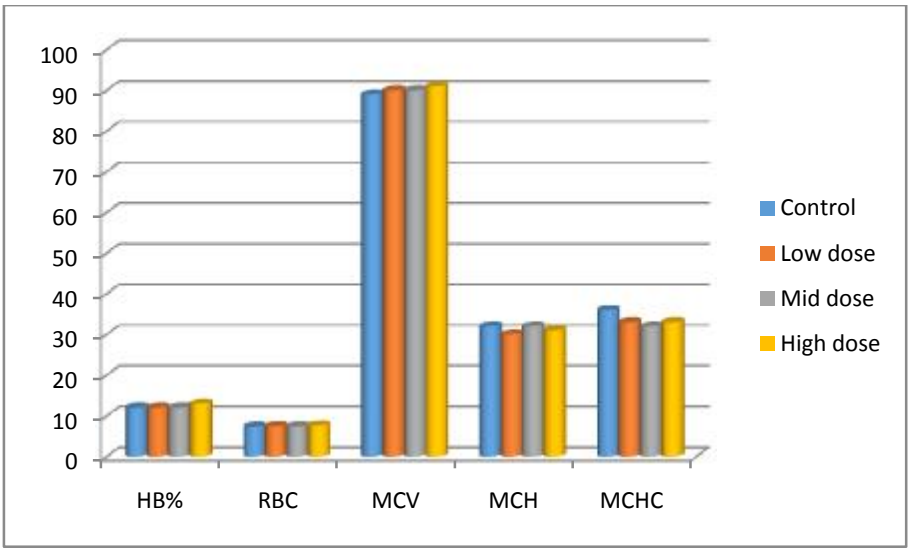


Chart 9: **Sub chronic toxicity - The mean value of T.WBC of control and treated groups of wistar albino rats exposed to Naga chenduram**

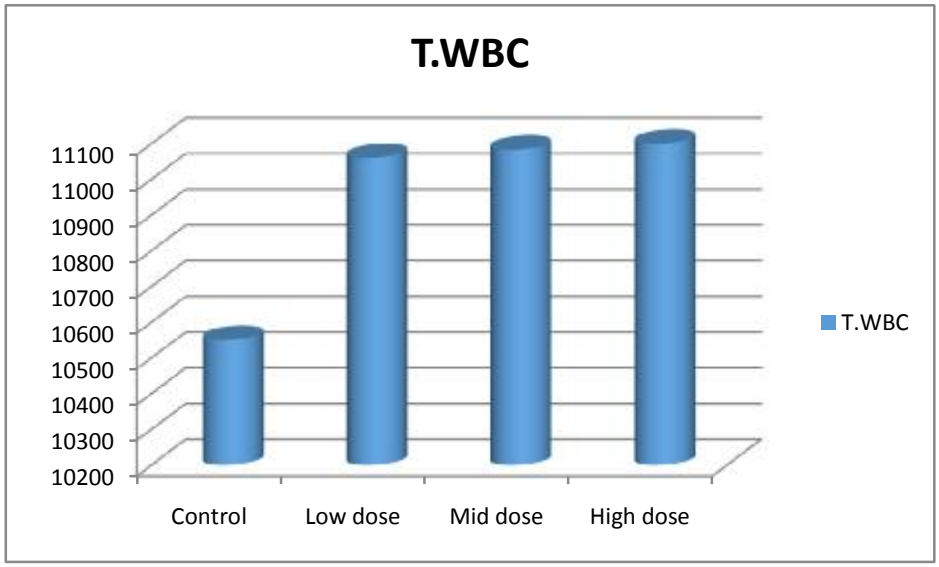



Chart 10: **Sub chronic toxicity - The mean value of Platelet of control and treated groups of wistar albino rats exposed to Naga chenduram**

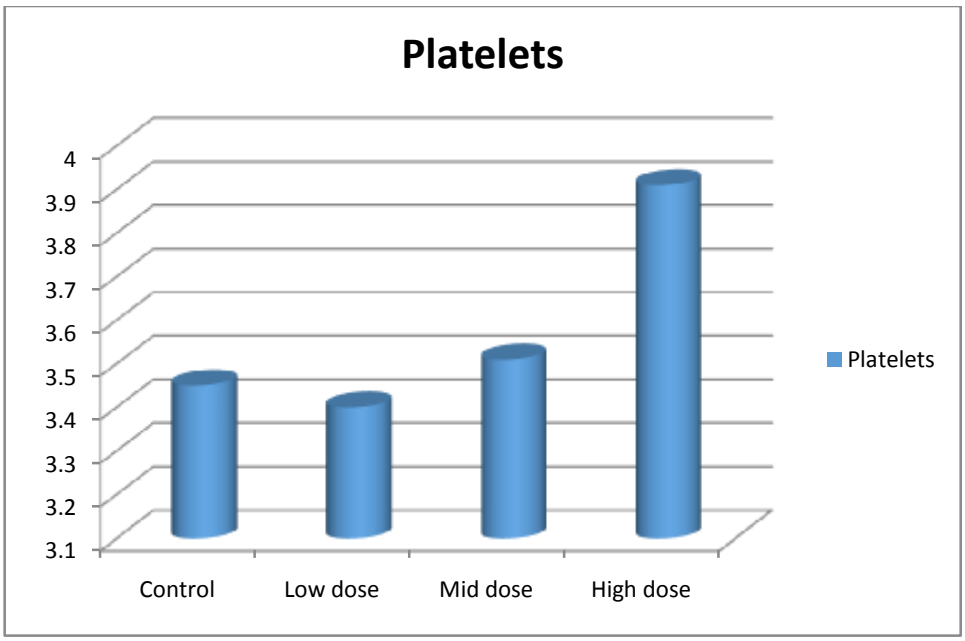


**Table 19: Sub chronic toxicity – Lipid profile of Wistar albino rats group exposed to *Naga chenduram***

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| **Totalcholesterol (mg/kg)** | 113±7.91 | 114±8.11 | 113±10.65 | 101±14.59 |
| **HDL(mg/dl)** | 41.33±6.97 | 36.5±1.87 | 45.66±2.06 | 39.16±4.87 |
| **LDL(mg/dl)** | 41.66±6.18 | 51.16±11.51 | 45.33±13.44 | 40.33±17.50 |
| **VLDL (mg/dl)** | 30.6±01.01 | 27±3.33 | 22.33±1.19 | 21.83±1.45 |
| **Triglycerides(mg/kg)** | 153±5.09 | 135±16.67 | 111±5.98 | 107 ±5.50 |

Values are mean of a 6 animals ± S.E.M (Dunnet's test)* p<0.05 ;**p<0.01.N=6

Chart 11: **Sub chronic toxicity - The mean value Lipid profile of control and treated groups of wistar albino rats exposed to Naga chenduram.**

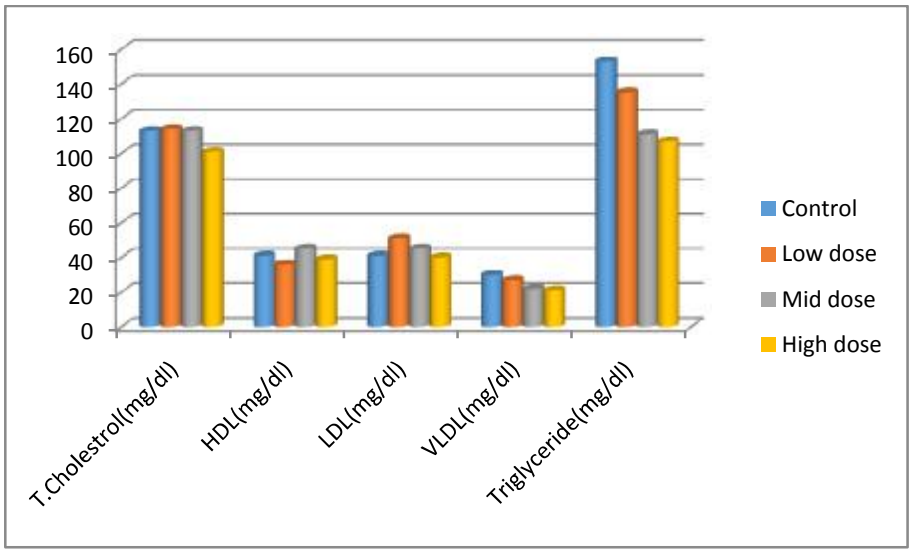


**Table20: Sub chronic toxicity – Total biliribin of Wistar albino rats group exposed to *Naga chenduram***

| Parameter | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| Total Bilirubin(mg/dl) | 0.6±0.06 | 0.66±0.16 | 0.68±0.21 | 0.55±0.10 |

Values are mean of a 6 animals ± S.E.M (Dunnet's test)* p<0.05;**p<0.01.N=6

Chart 12: **Sub chronic toxicity - The mean value of Total bilirubin of control and treated groups of wistar albino rats exposed to Naga chenduram**

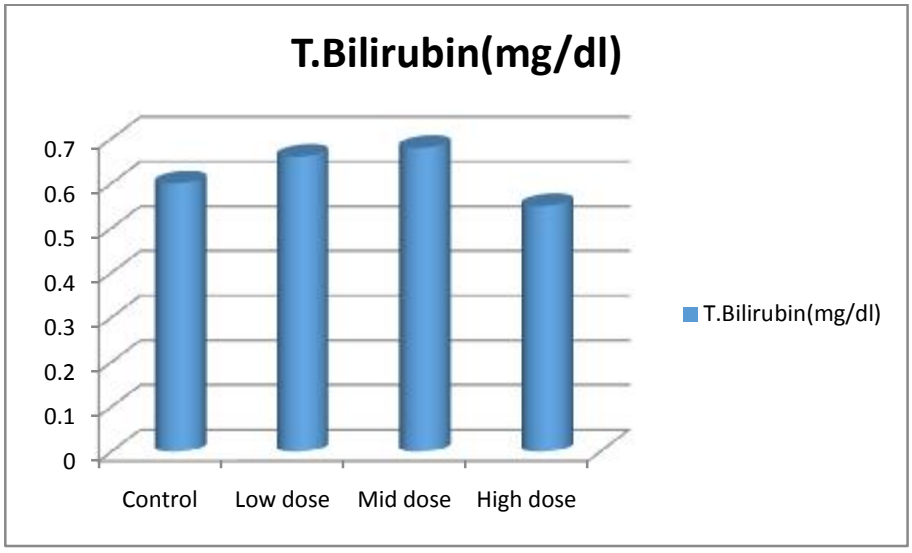



**Table 21**: **Sub chronic toxicity – SGOT, SGPT, ALP of Wistar albino rats group exposed to Naga chenduram**

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| SGOT(mg/dl) | 26±2.09 | 26.5±6.18 | 22.16±5.07 | 22.33±5.52 |
| SGPT(mg/dl) | 24.16±6.14 | 25±4.38 | 19±5.47 | 23.83±5.52 |
| ALP(mg/dl) | 74.5±9.56 | 67.83±15.25 | 77.16±11.61 | 60.5±9.93 |

Values are mean of a 6 animals ± S.E.M (Dunnet's test)* p<0.05;**p<0.01.N=6

Chart 13: **Sub chronic toxicity - The mean value of SGOT, SGPT, ALP of control and treated groups of wistar albino rats exposed to Naga chenduram**

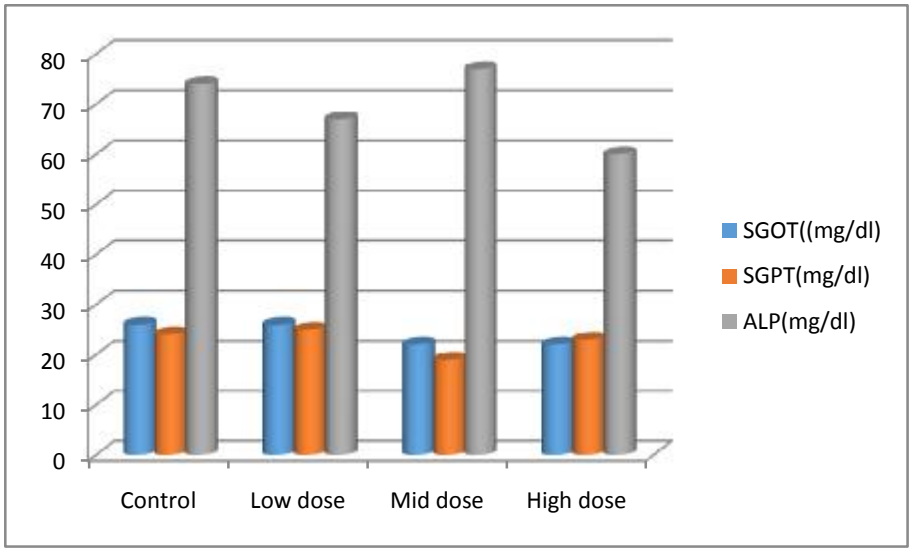



**Table22**: **Sub chronic toxicity - Blood glucose test of Wistar ano rats group exposed to Naga chenduram**

| Parameter | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| Blood gglucose(mg/dl) | 122.33±4.58 | 133.33±8.21 | 109.83±15.7 | 109.5±0.15.3 |

Values are mean of a 6 animals ± S.E.M (Dunnet's test)* $p<0.05$;**$p<0.01$.N=6

**Chart 15**: **Sub chronic toxicity - The mean value of Blood glucose of control and treated groups of wistar albino rats exposed to Naga chenduram**

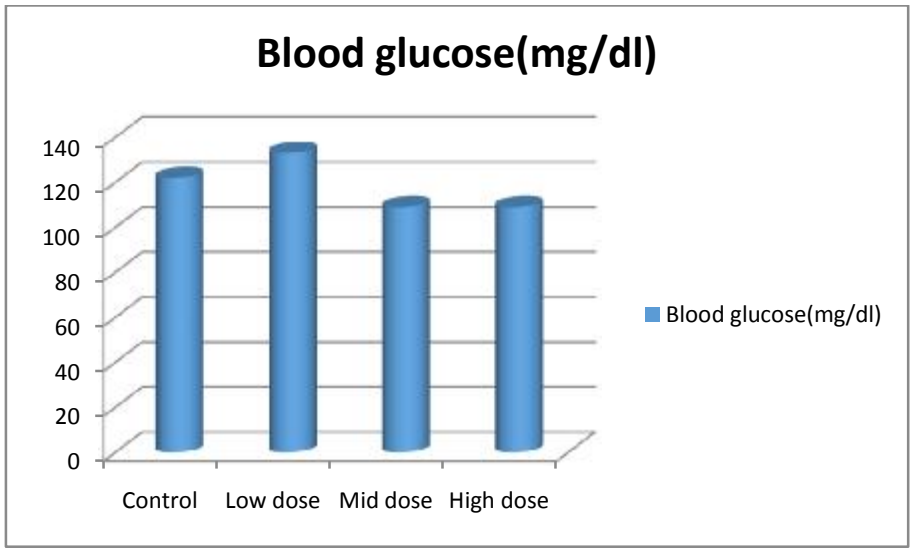



**Table23: Sub chronic toxicity - Urea test of Wistar albino rats group exposed to Naga chenduram**

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| Urea(mg/dl) | 30.66±7.76 | 28.66±3.32 | 28.33±9.30 | 30.83±4.07 |

Values are mean of a 6 animals ± S.E.M (Dunnet's test)* $p<0.05$;**$p<0.01$.N=6

**Chart 16: Sub chronic toxicity - The mean value of Urea of control and treated groups of wistar albino rats exposed to Naga chenduram**

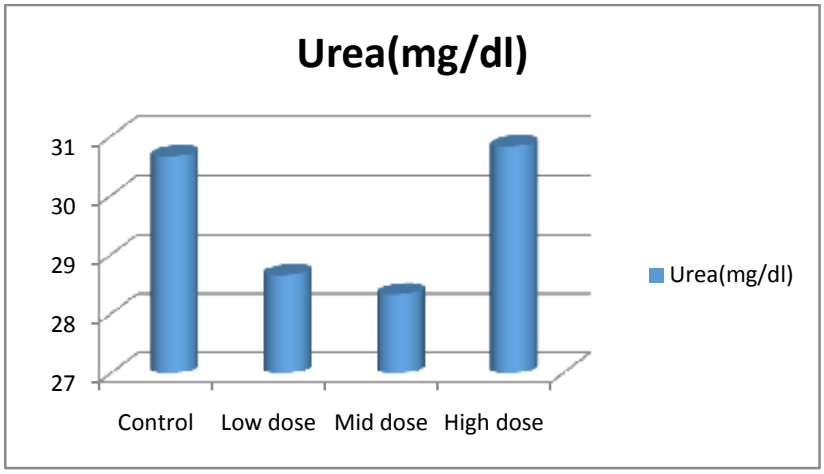
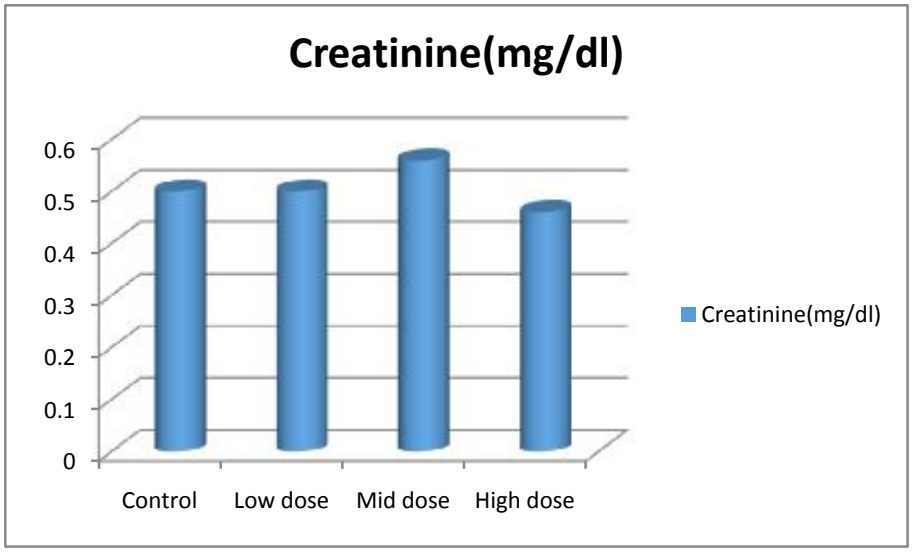



**Table24: Sub chronic toxicity – Creatinine test of Wistar albino rats group exposed to Naga chenduram**

| Parameters | Control | Low dose | Mid dose | High dose |
|---|---|---|---|---|
| Creatinine(mg/dl) | 0.5±0.14 | 0.5±0.14 | 0.56±0.16 | 0.46±0.18 |

Values are mean of a 6 animals ± S.E.M (Dunnet's test)* $p<0.05$;**$p<0.01$.N=6

**Chart 17: Sub chronic toxicity - The mean value of Creatinine of control and treated groups of wistar albino rats exposed to Naga chenduram**




**Results:**

**Interpretation of Sub chronic toxicity of *Naga chenduram***

All animals from control and all the treated dose groups survived throughout the dosing period of 90 days for sub chronic toxicity study. There was no significant change in the body weight for the control and treatment group throughout the dosing period of 90 days.

**Interpretation of hematological investigation**

The results of hematological investigations conducted on day 91$^{th}$ day revealed no significant changes in the hematological values when compared with those of respective controls. All the values were within the normal biological and laboratory limits.

**Interpretation of Biochemical investigation**

Results of Biochemical investigations conducted on days 91$^{th}$ and recorded in revealed the no significant changes in the values of different parameters studied when compared with those of respective controls. All the values were within the normal biological and laboratory limits.

**Intrepretation of histopathology:**

The vital organs such as liver, heart, kidneys, bone, and brain were removed from the test groups at the end of the study and carefully observed macroscopically to find any observable gross lesions compared with the control group and did not reveal any abnormal macroscopic changes. Cross pathological investigation was carried out and histopathology of vital organ reveled normal histological appearance when compared with the control.

**Table 25-Styptic activity of *naga chenduram***

| S.no | Parameters | Control | N.C(200mg/kg) | N.C(400mg/kg) |
|---|---|---|---|---|
| 1 | Bleeding time(s) | 90.23±1.06 | 86.21±0.25* | 82.12±0.09** |
| 2 | Clotting Time(s) | 119.20±0.46 | 115.01±0.67* | 102.21±1.20** |
| 3 | Prothrombin Time(s) | 24.12±0.21 | 24.20±0.36 | 18.28±0.11** |

n=6 Values are expresse das mean ±SEM analysis as done by students t test followed by dunnet's. P<0.05 when compared with control.(p-value <0.05 was taken as significant).

**Results:**

**Bleeding Time :( BT)**

In the vehicle treated control group the mean bleeding time was 90.23±1.06 seconds, while in the test drug treated group it was 85.12±0.12 seconds. The result shows significant effect, when compared to control group.

**Clotting time : (CT)**

The mean clotting time in this vehicle treated control group was 119.20±0.4 seconds and in the NC treated group the clotting time was 102.21± 1.2 seconds. The result shows significant effect, when compared to control group.

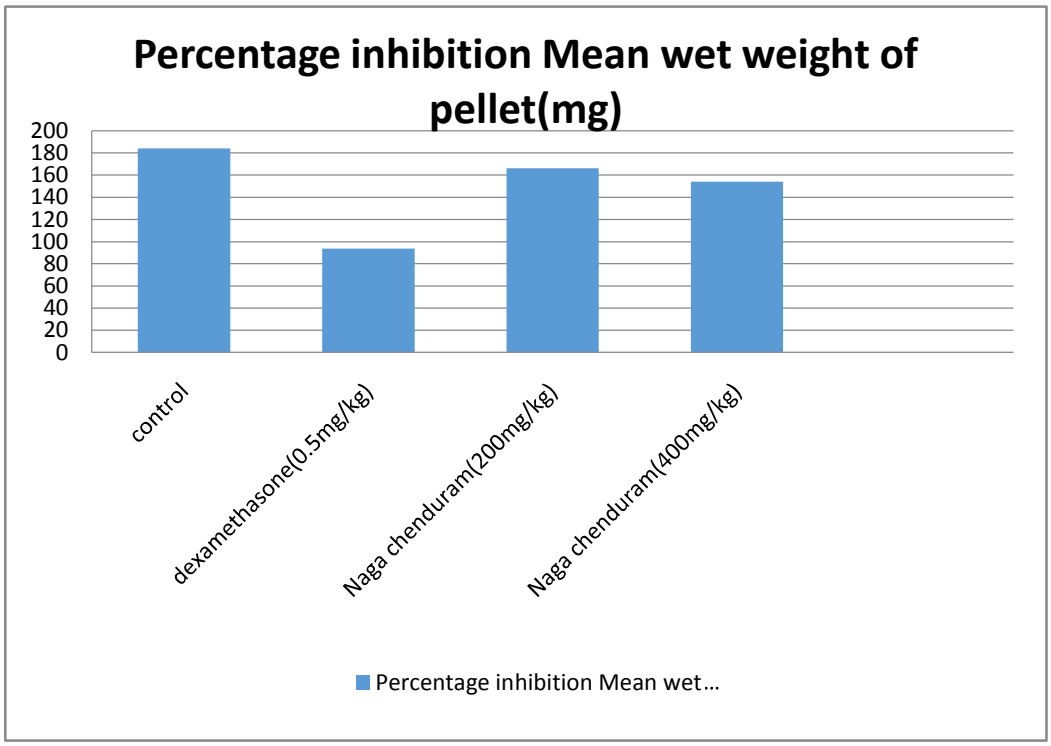
**Prothrombin time: (PT)**

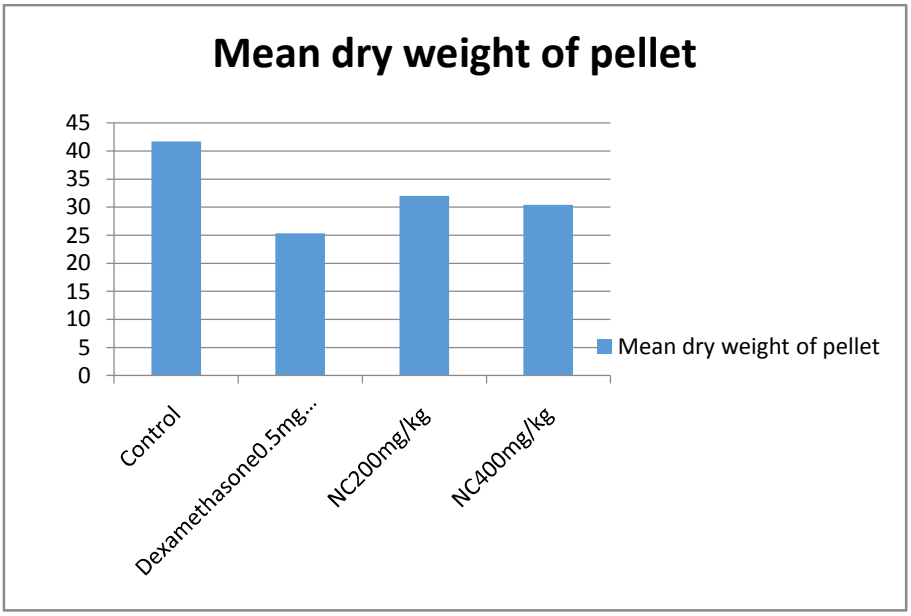
In the vehicle treated control group the mean prothrombin time 24.12±0.21 seconds and in the NC treated group it was 18.28±0.11 seconds. This shows that there is a significant difference between two groups indicating that there is an effect of NC treatment for the prothrombin time.

**Table 26.Anti-inflammotry activity of *naga chenduram* by Cotton pellet induced granuloma method.**

| Groups | Treatment | Mean wet weight of pellet(mg) | Percentage inhibition | Mean dry weight of pellet(mg) | Percentage inhibition |
|---|---|---|---|---|---|
| I | control | 184.5±3.08 | 0 | 41.66±1.37 | 0 |
| II | Standard drug(0.5mg/kg) | 93.5±2.35 | 49 | 25.36±0.78 | 54.21 |
| III | NC(200mg/kg) | 166.0±7.92* | 10.02 | 32.03±1.03* | 21.86 |
| IV | NC(400mg/kg) | 154.16±3.60** | 22 | 30.43±0.38** | 19.56 |

n=6, values are expressed as mean± SEM P<0.05 when compared with control. (P-value <0.05 was taken as significant).


Percentage inhibition Mean wet weight of pellet(mg)
200
180
160
140
120
100
80
60
40
20
0
control
dexamethasone(0.5mg/kg)
Naga chenduram(200mg/kg)
Naga chenduram(400mg/kg)
Percentage inhibition Mean wet…


Mean dry weight of pellet
45
40
35
30
25
20
15
10
5
0
Control
Dexamethasone0.5mg…
NC200mg/kg
NC400mg/kg
Mean dry weight of pellet

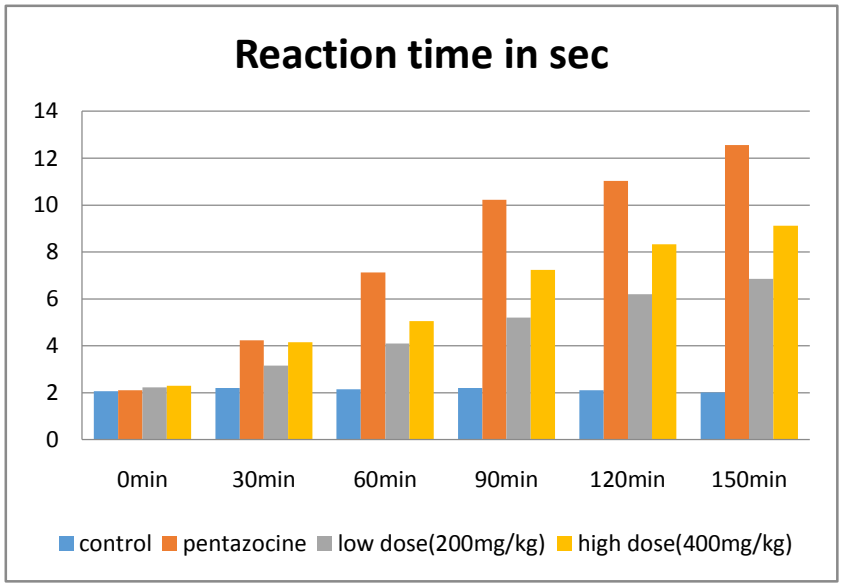
**Results:**

Anti-inflammatory effect of *Naga chenduram* were observed and found to be significant at the dose of 400mg/kg p.o. And compared with the vehicle of distilled water(10ml/kg p.o) and posses significant inhibition against cotton pellet induced granuloma in rats.

**Table 27.Analgesic activity of *Naga Chenduram* by Eddy's hot plate method.**

| Groups | Treament | Reaction time in sec | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0min | 30min | 60min | 90min | 120min | 150min |
| I | Control | 2.06±0.08 | 2.21±0.16 | 2.15±0.14 | 2.21±0.10 | 2.10±0.09 | 2.01±0.04 |
| II | Pentazocine (5mg/kg) | 2.1±0.17 | 4.23±0.27 | 7.13±0.15 | 10.23±0.38** | 11.03±0.08** | 12.56±0.37** |
| III | Low dose (200mg/kg) | 2.23±0.23 | 3.16±0.16 | 4.10±0.09 | 5.2±0.14 | 6.2±0.25 | 6.86±0.05* |
| IV | High dose 400mg/kg). | 2.03±0.05 | 4.15±0.15 | 5.05±0.08 | 7.23±0.27** | 8.33±0.37** | 9.11±0.16** |

n=6, values are expressed as mean± SEM P<0.05 when compared with control. The results were analyzed by ANOVA followed by Dunnet's test (p-value <0.05 was taken as significant)

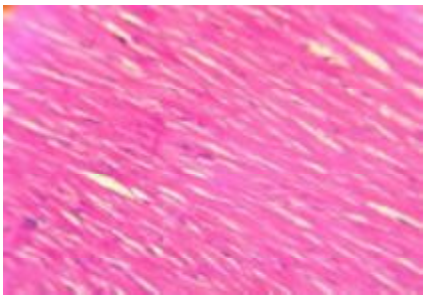


**Results:**

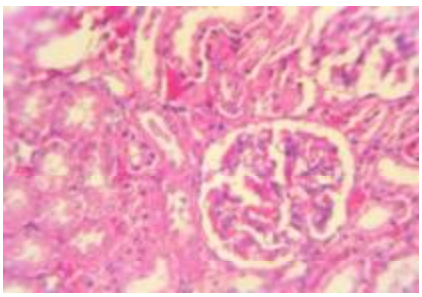
The analgesic activity of *Naga chenduram* determined by eddy's hot plate method respectively. The *naga chenduram* at the dose of 400mg/kg p.o exhibited marked analgesic effect as evidenced by significant increase in reaction time when compared to the vehicle (10ml/kg p.o)

**Sub acute toxicity -Histopathological studies of Naga chenduram on wistar albino rats.**

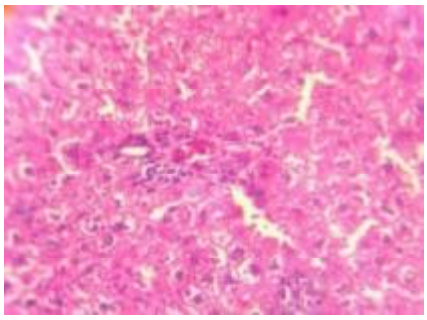
Control



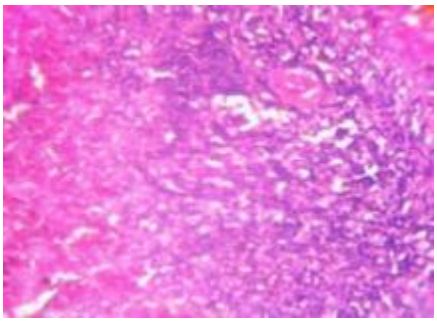
Heart



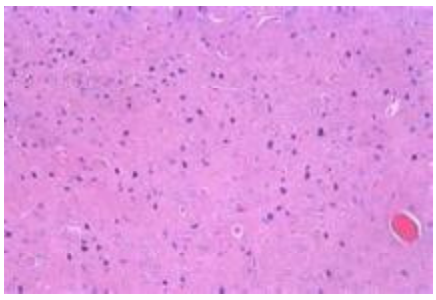
Kidney



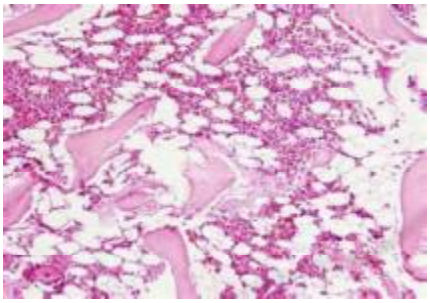
Liver



**Spleen**

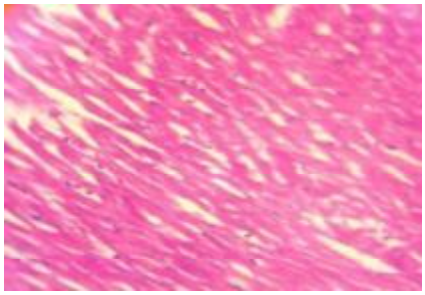


Brain

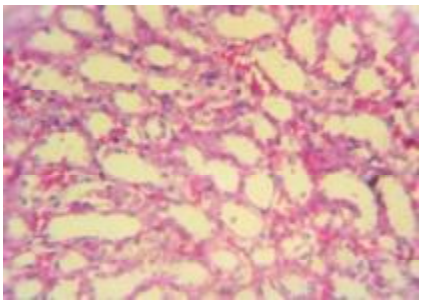


Bone

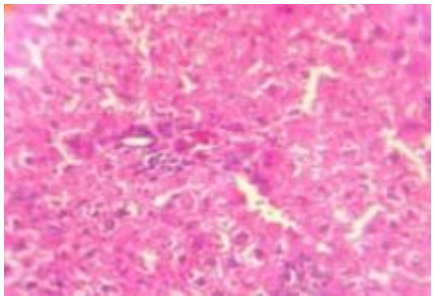
**Mid dose of *Naga chenduram for sub acute toxicity***



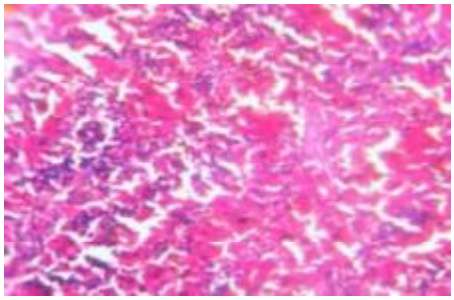
**Heart**



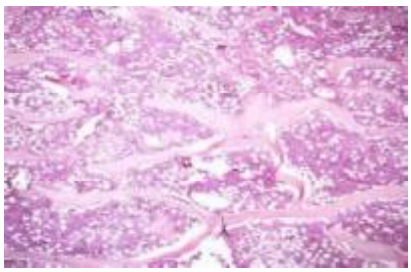
**Kidney**



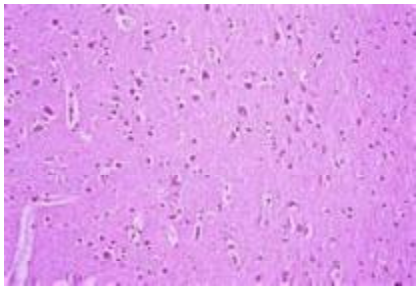
**Liver**



**Spleen**

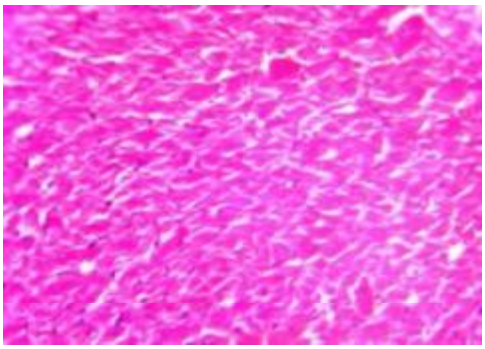


**Bone**

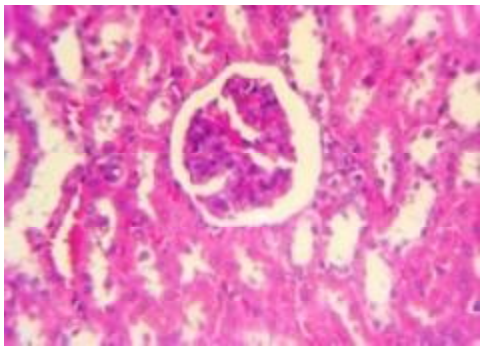


**Brain**

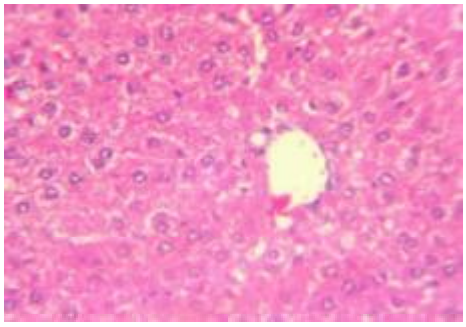
**High dose of *Naga chenduram* for sub acute toxicity**



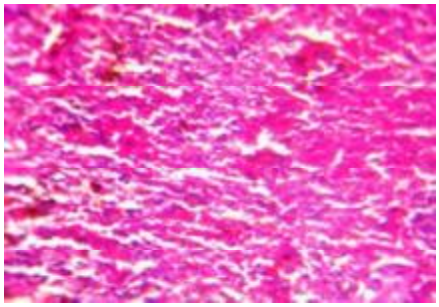
Heart



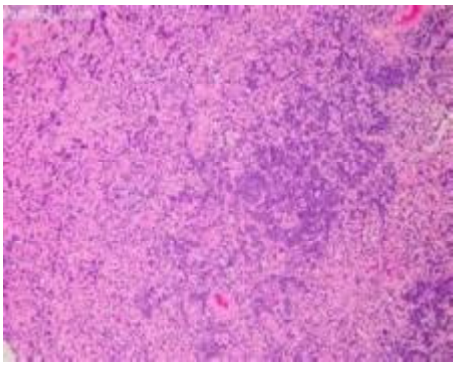
Kidney



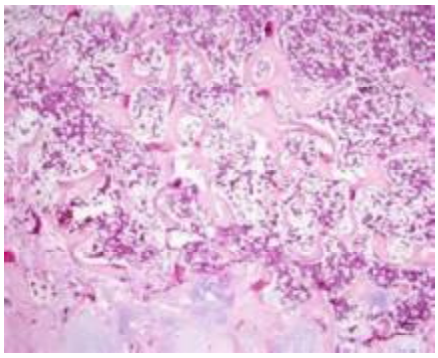
**Liver**



**Spleen**



**Brain**



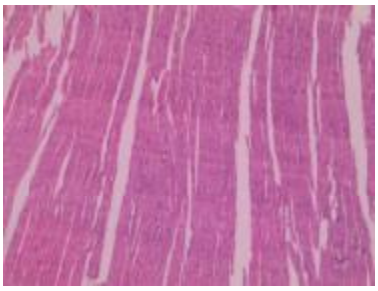
**Bone**

**Interpretation:**

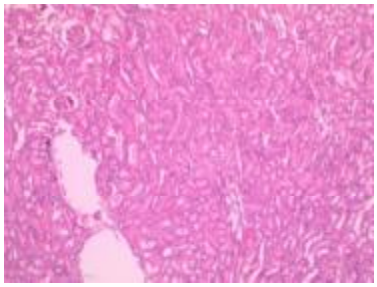
The above slides show the histopathology studies of the sub acute toxicity. There is no toxicological abnormality seen in the vital organs after administration of the drug Naga chenduram. Thus the safety of the drug is revealed so that it can be administered for long time without any side effects.

**Sub chronic toxicity -Histopathological studies of Naga chenduram on wistar albino rats.**

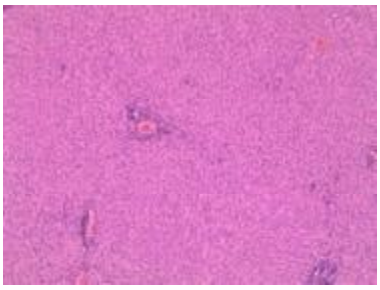
**Control:**



**Heart**

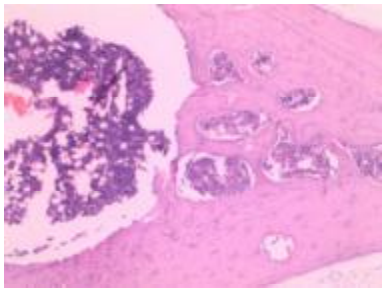


**Kidney**



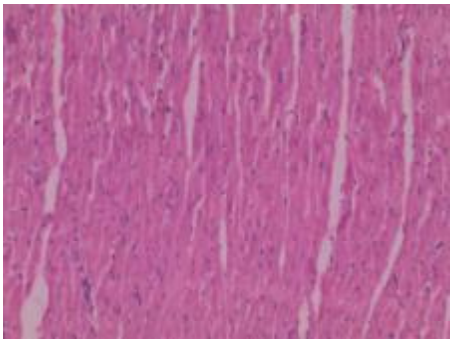
**Liver**

**Brain**

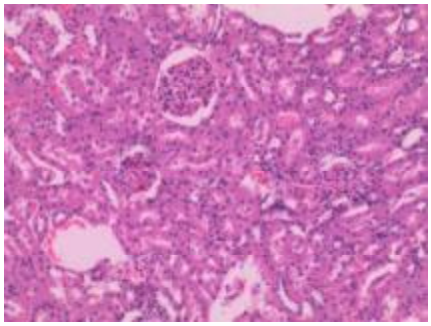


**Bone**

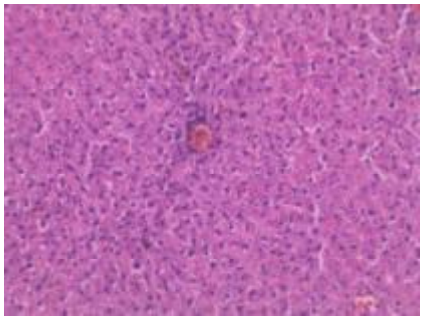
High dose of naga chenduram for sub chronic toxicity study



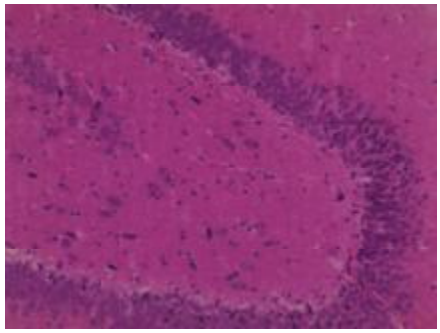
Heart



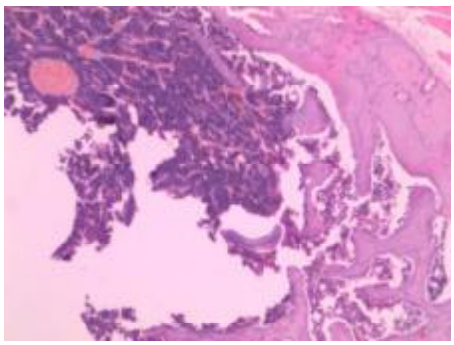
Kidney



Liver



Brain



**Bone**

**Interpretation of histopathology studies for sub chronic toxicity**

**Kidney**

- Some renal tubules appears hypertrophic in sample belongs to 6HM
- Interstitial connective tissue appears cohesive with distinct space in between
- Proximal and distal convoluted tubule appears normal
- No signs of cellular necrosis

**Heart**

- cardiomyocytes have a normal histological appear with uniform arrangement and thickness
- Appearance of cardiomyocyte was normal with dark nuclear region. The nuclei of muscle fibers appear central arrangement.

**Liver**

- Appearance of portal vein, bile duct and hepatic artery was normal
- Increased sinusoidal space were observed on centri lobular and midzonal region

**Brain**

- Arrangement of the neurons appears intact with no sings of degeneration or apoptotic changes in both the samples
- Cortex region showed normal neurons with polygonal to round cell bodies containing dense cytoplasm.

**Bone**

- Prominent segmented trabeculae separated from each other with normal thickness. Intertrabecular distance appears normal.
- Bone marrow cells were homogenously placed in marrow cavity

# *DISCUSSION*

## 9. DISCUSSION

The drug *Naga chenduram* was selected from the siddha literature "*Aathmarakshaamirtham ennum vaithiya sara sankiragam"* to evaluate the safety and its pharmacological activies (Styptic, anti – inflammatory, analgesic activity)

The ingredients of the test drug was identified and authenticated by Botanist.

The drug was prepared as per the procedure subjected to various studies such as qualitative, quantitative, toxicity and pharmacological activites. Qualitative analysis includes chemical analysis and physic- chemical properties of Naga chenduram.

Quantitative analysis included ICP-OES and HR SEM analysis to reveal its potency and effectiveness against the disease.

From the above analysis we came to know the presence of active ingredients responsible for its activity.

**Literary collections:**

Literary collections include drug review, which consist of both Botanical aspects, Gunapadam aspect, Pharmacological review that supported the study.

**Chemical analysis:**

Chemical analysis of the drug *Naga chenduram* reveals that the presence of Chloride, Magnesium, Sodium, Potassium, Calcium.

**Potassium:**

It is useful in maintaining the tissues in digestive tract which reduces inflammation of stomach and intestine.

Potassium absorption and secretion by the human intestine.

**Magnesium:**

It is focusing on repairing digestive system and preventing constipation.

**Chloride:**

It is an essential part of digestive (stomach) juices.

Chloride is a efficient in increasing defecation frequency and in softening stool.

The sodium and the chloride components of salt contribute to digestion.

**Calcium:**

Taking calcium supplements will help in strengthening the veins and facilitating easy bowel movements.

Calcium is necessary for the muscle contraction which helps to reduce muscle cramps during constipation.

**Sodium**

The sodium components of salt contribute to digestion.

**Physico chemical analysis:**

The acid insoluble ash value denotes the drug quality. The drug possess within normal value (5.43 %) of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.

The loss on drying value of Naga chenduram was found to be 0.53 % w/w, hence the drug will not lose much of its volume on exposure to the atmospheric air at room temperature.

In ICP-OES study, heavy metals were found below detection limit in Naga chenduram, calcium, sodium, phosphorus, potassium were present.

In HR SEM analysis, the particle size of Naga chenduram showed irregular morphology and distributed in near nano range. Naga chenduram has particle size of 1 to 2 μ.This ensures the absorption of the drug was more active and the drug have increased bio- availability.

**Toxicological studies:**

This study reveals that no significant toxic effect of the drug *Naga chenduram* up to the higher dose level 2000mg/kg in acute oral toxicity and also sub acute toxicity and sub chronic toxicity has no toxic effects from the results. Therefore the *Naga chenduramcan* be classified under the category of drug with non-toxic.

**Pharmacological studies:**

The Pharmacological study the experimental data showed that the *Naga chenduram* has Styptic, Anti- inflammatory, Analgesic activity and the results are as follows,

**Styptic activity:**

**Bleeding Time: (BT)**

In the vehicle treated control group the mean bleeding time was 90.23±1.06 seconds, while in the test drug treated group it was 85.12±0.12 seconds. The result shows significant effect, when compared to control group.

**Clotting time: (CT)**

The mean clotting time in this vehicle treated control group was 119.20±0.4 seconds and in the NC treated group the clotting time was 102.21± 1.2 seconds. The result shows significant effect, when compared to control group.

**Prothrombin time: (PT)**

In the vehicle treated control group the mean prothrombin time 24.12±0.21 seconds and in the NC treated group it was 18.28±0.11 seconds. This shows that there is a significant difference between two groups indicating that there is an effect of NC treatment for the prothrombin time.

**Anti - inflammatory activity:**

Anti-inflammatory effect of *Naga chenduram* were observed and found to be significant at the dose of 400mg/kg p.o. And compared with the vehicle of distilled water(10ml/kg p.o) and posses significant inhibition against cotton pellet induced granuloma in rats.

**Analgesic activity:**

The analgesic activity of *Naga chenduram* determined by eddy's hot plate method respectively. The naga chenduram at the dose of 400mg/kg p.o exhibited marked analgesic effect as evidenced by significant increase in reaction time when compared to the vehicle (10ml/kg p.o)

# *SUMMARY*

## 10. SUMMARY

- The literary evidence of the drug *Naga chenduram* strongly support that it possesses Styptic, Anti-inflammatory and Analgesic activity for that purpose it has been selected for this study.

- The qualitative chemical analysis was done at Biochemistry lab, NIS. Chemical analysis of the drug *naga chenduram* reveals that the presence of potassium, Sodium, Chloride, Magnesium, Calcium and Alkaloid.

- Preclinical evaluation of acute, sub-acute toxicity studies the drug was carried out as per OECD guideline in from animal house, K.k College of pharmacy, Gerugambakkam, Chennai and sub-chronic tocicity studies the drug was carried out as per OECD guideline in from animal house, NIS, Chennai. This study reveals no significant toxic effect of the *Naga chenduram* upto the higher dose level 2000mg/kg used in this study.

- Pharmacological study (Styptic, Anti- inflammatory, Analgesic activity) of the drug was carried out as per OECD guideline in , k.k college of pharmacy, gerugambakkam, Chennai. In the pharmacological studies, the drug *Naga chenduram* exhibits significant Styptic activity, Anti- inflammatory, Analgesic activity.

- From the results and the statistical analysis it is proved that the drug *Naga chenduram* is significant of Styptic activity, Anti- inflammatory, Analgesic activity in the management of hemorrhoids and ano rectal disorders.

# *CONCLUSION*

## 11. CONCLUSION

From the literature evidence, Physico chemical analysis, chemical analysis, Toxicological evaluation and Pharmacological studies, the drug *Naga chenduram* has Styptic activity, Anti- inflammatory, Analgesic activity. It is concluded that the drug *Naga chenduram* can be used in the management of hemorrhoids and the related ano rectal disorders.

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# *ANNEXURE*